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ERRATA, VOL. XXXI

- No. 4, page 297, lines 4 and 7. *Nitrate* should read *nitrite*.
 No. 8, page 717, line 32. *High* should read *low*.
 No. 9, page 768, line 18. 763-768 should read 769-777.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXI

JANUARY, 1948

NUMBER 1

STUDIES OF THE GROWTH AND BLOOD COMPOSITION OF DAIRY CALVES FED REMADE SKIM MILK AFTER THREE DAYS OF AGE¹

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The common method of feeding dairy calves for the first few weeks consists of feeding whole milk or partially skimmed milk for a limited time and then changing to skim milk. Other methods reported (3, 4, 10) involve changing abruptly to skim milk after the colostrum period and have proved satisfactory when the skim milk has been supplemented with vitamins A and D. Less success has been reported, however, with feeding remade skim milk. Morrow (11), in comparing fresh skim milk with remade skim milk, found that calves fed remade skim milk did not make as good growth as those fed fresh skim milk. Digestive disturbances also were noted in those calves fed the remade skim milk. Knott *et al.* (6) fed remade skim milk after 2 to 6 weeks of age and thereafter fed skim milk powder in the grain ration; they secured normal growth.

The partial success with feeding remade skim milk indicated the need for additional study of this method of feeding. The effects of this method would be expected to be exhibited, first, in the growth of the animals. The low-fat intake of the ration indicated a study of the blood fat content. Allen (2) has reported that the blood fat of dairy cattle is closely associated with feeding conditions. He reported that the blood of the newborn calf contains only a trace of fat, but the fat increases rapidly with the ingestion of food. This method of feeding also suggested a study of the blood carotene and vitamin A, since the skim milk must be supplemented with carotene or vitamin A.

EXPERIMENTAL PROCEDURE

Seven Holstein and six Jersey male calves from the Virginia Polytechnic Institute dairy herd were used for this experiment. The seven Holstein calves and five of the Jersey calves were removed from their dams on the

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¹Data taken from thesis presented by L. R. Arrington as partial requirement for degree of Master of Science in Dairy Husbandry, 1941.

²Now at the University of Florida, College of Agriculture.

third day after birth and placed on the experimental ration. One of the Jersey calves was 10 days old when placed on the experiment and had received whole milk until that time. Each calf was housed in an individual stall throughout the experiment. An additional Holstein calf was used as a control, being handled in the same manner as the others except that fresh whole milk was fed in place of remade skim milk.

The experimental ration of remade skim milk was prepared by mixing 1 lb. of skim milk powder (spray process) with 9 lb. of warm water. This was fed at the rate of 1 lb. of milk daily per 10 lb. of body weight. A grain mixture was supplied as soon as the calves would eat it. The mixture consisted of 200 lb. of corn, 100 lb. of wheat, 250 lb. of whole oats, 250 lb. of wheat bran, 100 lb. of distillers grain, 100 lb. of linseed meal, 10 lb. of steamed bone meal, and 10 lb. of salt. Chopped red clover and alfalfa hay was supplied *ad libitum* as early as the calves would eat it.

One group of calves was fed a carotene concentrate;³ the other group was fed cod-liver oil as the supplement. Those calves receiving the carotene supplement were given 20 drops of viosterol daily as a protection against vitamin D deficiency. The rate of carotene feeding was adjusted weekly with the increase in body weight. These concentrates were fed by mixing with the milk at the evening feeding.

Body weight and height at withers were taken at 3 days of age, when each calf was placed on the experiment, and each week thereafter, and blood fat determinations were made using the method described by Allen (1). Blood carotene determinations also were made weekly by the method of Moore (7), and blood vitamin A determinations were made weekly on six of the calves by the method of Kimble (5).

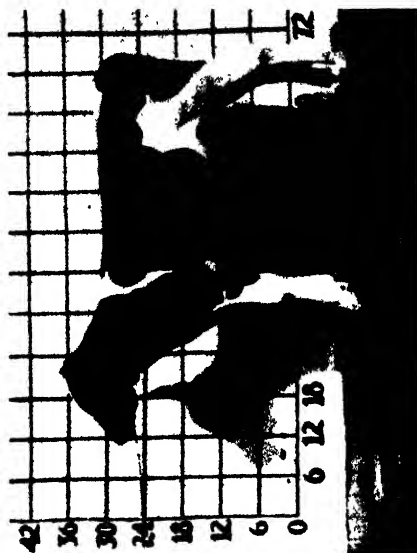
RESULTS

Six of the seven Holstein calves lived through the experimental period. Only one of the Jerseys (no. 812) lived through the experimental period, but this one was 10 days old when placed on experiment and had received whole milk until that time. Another Jersey calf (no. 813) lived until 14 weeks of age and died of bloat. Gain in body weight for this calf was slightly below normal. Since four of the six Jersey calves died within 2 to 4 weeks following the change to remade skim milk, they evidently could not withstand the abrupt change to the experimental ration or did not have the reserve to carry them over until they consumed feed other than milk. In each case where the calf died, scours occurred, and there was a steady loss in weight. Scours occurred in some of the other calves also, and it became necessary to reduce the milk intake. After the first 2 to 3 weeks, scours was much less prevalent, and animals appeared to be in better general health. There were no evidences of vitamin A or D deficiency.

³ A commercial carotene preparation, Carotene—Type P-25, prepared and supplied by General Biochemicals, Inc.



Calf no. 819—Holstein fed carotene and viosterol. Age, 16 weeks; weight, 248 lb.



Calf no. 821—Holstein fed cod-liver oil. Age, 16 weeks; weight, 240 lb.

FIG. 1. Typical calves from each group receiving a supplement.

Table 1 shows the weekly weight of each calf throughout the experimental period. The Holsteins showed little or no gain for 2 or 3 weeks following the change to the experimental ration, and the Jerseys lost weight during this time. The retarded rate of gain is attributed to the low T.D.N. intake of the ration, since increases were nearly normal soon after grain and hay were consumed regularly. The intake of nutrients was below the requirement according to the Morrison Standard (9) until grain and hay were taken.

With the normal intake of grain and hay at about 6 weeks of age, increases in body weight were almost normal, and at 16 weeks of age the calves were practically normal size according to figures of Ragsdale (12).

Gains in height at withers were slightly below the normal rate (12) for the first few weeks. After about 3 weeks the Holsteins showed a normal increase in height, and after about 5 weeks the increase for the Jerseys compared favorably with the normal. There was no apparent difference in body weight and height at withers between the group fed carotene and the group fed cod-liver oil. Calves no. 819 and 821 were selected as typical calves from each group. Figure 1 shows a photograph of each.

Table 2 shows the weekly blood fat content of each calf. There were variations from week to week and there were differences among the animals, but there was no significant difference between the group fed carotene and the group fed cod-liver oil. Blood fat of the experimental calves was lower than that of the control calf fed whole milk. With the increase in intake of grain and hay, blood fat increased.

Blood carotene levels in those calves fed the carotene supplement were well above those reported as minimum requirements (8). The amount of blood carotene was considerably higher in this group than in the group fed cod-liver oil (table 3). There were slight variations from week to week in each group, but a general trend to a higher level toward the end of the experiment, when the calves were eating more hay, was apparent.

Blood vitamin A determinations were made on only six of the experimental animals, including the control. The plasma concentrations of those calves fed cod-liver oil as a supplement were higher than those of either the carotene group or the control calf (table 4). Concentrations for the control calf ranged intermediate between those of the cod-liver oil group and the carotene group.

SUMMARY

Seven Holstein and six Jersey male calves were fed an experimental ration of remade skim milk supplemented with vitamin A and D in the form of carotene and viosterol or cod-liver oil. The seven Holstein calves and five of the Jersey calves were placed on the experimental ration at 3 days of age. One of the Jersey calves received whole milk until 10 days of age. Grain and hay were fed as soon as the calves would eat it.

TABLE 1
Body weight of experimental calves

No. of calf	Breed	3rd day	Age (weeks)																
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
817*	H	75	75	77	84	90	103	113	125	140	145	153	179	193	207	219	237		
(lb.)																			
Carotene group																			
810	H	100	93	97	91	Died	62	70	76	85	90	103	109	119	132	145	167		
812	J	45	56	54	55	55													
814	J	45	47	45	Died		124	125	135	149	168	183	197	211	224				
815	H	92	93	99	108	112	119	127	132	143	150	163	171	182	198	206	210	235	
819	H	102	102	104	108	119	127	132	143	150	163	171	182	198	206	210	235	248	
820	H	97	97	98	103	108	112	120	127	136	147	157	164	177	186	192	209	228	
822	H	90	93	93	100	102	106	113	123	125	143	152	Experiment terminated						
823	J	47	45	44	43	41	Died												
824	J	51	50	47	41	Died													
Cod liver oil group																			
813	J	55	52	50	51	55	62	64	74	82	94	96	105	115	128	Died of bloat			
816	J	50	44	43	Died														
818	H	84	82	80	85	91	99	113	120	133	142	153	164	179	185	194			
821	H	102	103	104	106	112	120	126	137	145	154	165	180	190	199	200	227	240	

*Control calf.

TABLE 2
Fat content of blood plasma

No. of calf	Breed	3rd day	Age (weeks)																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16				
			(mg./ml.)																			
817*	H	29	49	57	78		87	140	135	Carotene group												
												123	154	149	164	146	142					
812	J.								76	39	32	47	48	86	87	118	69					
815	H			15	16		14	31	35	55	46	83	70	52	29	50						
819	H		22	42		24	24	23	40	35	83	65	128	144	178	218	208	155				
820	H	40	27		11	11	23	38	24	44	44	93	99	158	189	190	132	133				
822	H		24	25	34	65	69	105	106	89	49	64	Experiment terminated									
823	J	47	110	78			Died															
824	J	20	63				Died															
			Cod-liver oil group																			
813	J							53	27	20	38	22	53	39			Died (of blost)					
816	J	24	20	10	Died																	
818	H	25	19	20	22	16	29	24	31	31	31	36	46	51	75	129	131					
821	H	50	26		32	23	12	15	26	27	54	110	87	157	180	173	126	119				

*Control calf.

TABLE 3
Carotene content of blood plasma

No. of calf	Breed	3rd day	Age (weeks)															
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
817*	H	0.26	0.16	0.15	0.08		0.11	0.21	0.08	($\mu\text{g./ml.}$)	0.16	0.22	0.54	0.48	0.49	0.45	0.59	0.62
Carotene group																		
812	J						0.15	0.39	0.39	0.23	0.54	0.40	0.41	0.74	0.58	0.42	0.85	0.59
815	H	0.34	0.10	0.20	0.11	0.24	0.25	0.25	0.45	0.26	0.11	0.48	0.45	0.31				
819	H					0.42	0.45	0.49	0.44	0.44	0.42	0.66	0.92	1.34	1.03	1.03	1.12	0.76
820	H	0.12	0.11	0.30	0.18			0.38	0.35	0.35	0.50	0.58	0.84	0.72	0.70	0.80	0.49	0.47
822	H					0.27	0.26	0.41	0.42	0.46	0.45	0.15	0.16	Experiment terminated				
Cod-liver oil group																		
813	J			0.10	0.20	0.25	0.15	0.30	0.28	0.14	0.34	0.34	0.21	0.21	Died (of bloat)			
818	H	0.05	0	0.09	0.03		0.04	0.05	0.15	0.16	0.16	0.16	0.30	0.47	0.61			
821	H	0.23	0.07	0.03		0.03	0.04	0.23	0.12	0.16	0.14	0.14	0.11	0.12	0.08	0.14		

* Control calf.

TABLE 4
Blood vitamin A

No. of Breed calf	Age (weeks)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
817* H									0.08	0.10		0.07	0.13	0.18	0.13	0.10
(μg./ml.)																
Carotene group																
819 H			0.08	0.05		0.04	0.06	0.08	0.05			0.04	0.07	0.06	0.06	0.08
820 H			0.09	0.07		0.05	0.07	0.07	0.07	0.06	0.04	0.03	0.05	0.05	0.10	0.09
822 H	0.15	0.06	0.03		0.03	0.03	0.04	0.02	0.03	Experiment terminated						
Cod-liver oil group																
818 H										0.14	0.21	0.23	0.18	0.13	0.15	
821 H			0.16	0.10		0.17	0.17	0.12	0.11	0.10	0.11	0.16	0.17	0.15	0.19	0.18

* Control calf.

Six of the seven Holstein calves lived through the experimental period of about 16 weeks. Growth rates were below normal for the first few weeks, but Holstein calves were practically normal size at 16 weeks of age. Four of the six Jersey calves died within 2 to 4 weeks following the change to the experimental ration. One of the other Jerseys died at 14 weeks of age from bloat, and only one Jersey lived through the 16-week experimental period. This one received whole milk until 10 days of age. There was no difference in growth rates between the groups fed the different vitamin supplements.

Blood fat content was lower in calves fed the experimental ration than that of the control calf fed whole milk. As the quantity of grain and hay consumed increased, the blood fat increased.

Blood carotene was higher in calves receiving the carotene supplement than that of the control calf on whole milk or that of the experimental calves receiving cod-liver oil as the supplement.

Blood vitamin A of calves fed cod-liver oil was higher than that of the control calf or that of the group receiving carotene as the supplement.

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THE EFFECT OF PENICILLIN UPON THE LIVABILITY, GLYCOLYSIS, AND BACTERIAL CONTENT OF BOVINE SEMEN^{1,2}

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One of the most important problems in the artificial breeding of dairy cattle at the present time is the control of the bacterial flora of diluted bull semen. While present-day techniques in the collection of bull semen under routine artificial breeding conditions tend to reduce bacterial contamination, it is still impossible to obtain semen which is absolutely free of bacteria. Unless adequate precautions are taken in the preparation and handling of the diluted semen, further contamination may occur. In addition, both undiluted and diluted semen serve as excellent media for bacterial growth.

It is believed that the semen of the bull may serve as a means of transmitting certain genital infections which are related to fertility problems in a dairy herd. The widespread use of artificial breeding has greatly magnified the seriousness of this problem.

On the basis of the foregoing facts, the addition to semen of an anti-bacterial substance which does not exert an injurious effect on the spermatozoa would be of value in artificial breeding under field conditions.

As early as 1917, Ivanov (3) recognized and investigated the possibility of controlling the spread of infection by adding certain chemical substances, namely, ethyl alcohol, atoxyl, and salvarsan, to contaminated semen. In 1940 Shettles (8) reported that the survival and activity of human spermatozoa were not reduced by sulfanilamide or sulfapyridine in concentrations as high as 160 mg. per 100 ml. of the Baker's fluid used for diluting purposes. However, Knodt and Salisbury (4) were the first investigators to study the feasibility of using certain bacteriostatic compounds to control bacterial growth in bull semen. Using sulfanilamide at levels ranging from 0 to 1,000 mg. per 100 ml. of yolk-citrate diluter, they found that levels of 200 mg. and over controlled the growth of bacteria in stored diluted semen (20 days' storage at 5° C.). They concluded that 300 mg. of sulfa-

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nilamide per 100 ml. of diluter was the most favorable level since it not only prevented bacterial growth but also brought about a significant increase in spermatozoan livability. Further studies by these workers (6) more recently have shown that the addition of sulfanilamide to diluter at this optimal level gave an increase in the fertility of bull semen used in the routine artificial breeding of dairy cows. They attributed the beneficial effects of sulfanilamide on fertility to changes in the metabolism of the spermatozoa rather than to the control of bacterial growth alone.

Recently the possibility of using other antibacterial agents to prevent bacterial growth in bull semen has been reported. In attempts to produce a synthetic diluting medium which would be superior to the egg yolk-buffer diluters commonly used at the present time, Phillips and Spitzer (5) noticed considerable growth of bacteria in some of the preparations. After studying the effect of various antibacterial substances upon the motility of spermatozoa during storage, they recommended that 0.03 per cent of sulfathalidine, sulfasuxidine, or streptomycin be added to their newly developed lipid-glucose-buffer-gum (LGB) diluter to control bacterial contamination. They also mentioned that penicillin was not deleterious to spermatozoan motility but did not recommend its use in their LGB diluter.

Since penicillin is recognized as one of the most effective antibiotic agents in treating a variety of infections and is unique because of low tissue toxicity, it seemed desirable to study its use in bovine semen. Thus laboratory and field experiments were undertaken to determine the effects of penicillin upon the bacterial content of diluted bovine semen and its influence on the livability, metabolism, and fertility of the spermatozoa. The effect of penicillin upon fertility still is under investigation and will be reported later.

EXPERIMENTAL

Effect of penicillin upon spermatozoan livability. Preliminary studies to determine the effect of penicillin upon spermatozoan livability indicated that levels of 2,500 up to 10,000 Oxford units of penicillin per ml. of diluted semen were definitely detrimental. Thus, penicillin was added to 12 samples of bull semen at the rate of 0, 250, 500, 750, 1,000, 1,250, 1,500, and 2,000 Oxford units per ml. of diluted semen. The 12 ejaculates were diluted at the constant rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. This dilution was selected because it represented the average dilution rate being used by the artificial breeding cooperatives in Pennsylvania at the time the experiment was designed. The yolk-citrate diluter was composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g. of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in 100 ml. of water distilled over glass. When the design of the experiment involved the addition of penicillin, the desired amount of this substance was dissolved in the sodium citrate solution and mixed with the egg yolk in order to ensure the preparation of a diluter which would have a 1:1 ratio of yolk to buffer.

To test the effect of penicillin upon spermatozoan livability, the diluted semen was stored at 4.5°C. and the percentages of actively motile spermatozoa were determined every 2 days for a period of 20 days. At 0, 8, and 16 days of storage, subsamples were taken for glucose and lactic acid determinations, as well as for bacterial counts and penicillin assays.

The 12 ejaculates studied had a mean concentration of 1,147,000 spermatozoa per cubic millimeter, a mean motility of 70 per cent active spermatozoa, and a mean methylene blue reduction time of 7.2 minutes (range 5.0 to 10.0 minutes).

The results of the motility observations are shown in table 1. Each figure represents a mean of 12 ejaculates. Analysis of variance involving a total of 960 motility estimations showed no significant differences in livability between the 0, 250, 500, and 750 unit levels of penicillin. The 1,000,

TABLE 1
The effect of penicillin upon the livability of spermatozoa

Penicillin units per ml. diluted semen	Per cent motile spermatozoa (12 ejaculates)					
	Before storage	After storage at 4.5° C. for				
		4 days	8 days	12 days	16 days	20 days
Control	70	52	41	31	17	11
250	70	52	40	30	16	11
500	70	52	40	27	17	7
750	70	54	40	27	16	7
1000	70	52	39	26	16	6
1250	70	51	37	25	14	4
1500	70	47	33	22	12	4
2000	70	45	32	21	10	2

1,250, 1,500, and 2,000 unit levels of penicillin brought about a highly significant ($P = < 0.01$) decline in the ability of the spermatozoa to maintain motility as compared to the untreated controls. Highly significant differences between ejaculates and between storage intervals were found, as well as highly significant ejaculate \times treatment and storage interval \times ejaculate interactions. Apparently the various ejaculates responded differently to the several levels of penicillin in their livability during storage.

An examination of the mean motility observations for each treatment indicated that there was a more or less uniform decline in livability with increased amounts of penicillin. Thus, the relationship between spermatozoan livability and level of penicillin also was studied by means of regression. Both highly significant linear and curvilinear regressions were obtained (fig. 1). However, a test of significance of departure from linearity showed that the linear regression represented the livability data more accurately. Based on linear regression, the mean percentage of motile spermatozoa during 20 days' storage decreased by 0.9 per cent for each additional 250 units of penicillin as compared to the untreated controls.

Since in routine artificial breeding practically all semen is used by the time it is 6 days of age, an analysis of variance was made which included only the motility data for 2, 4, and 6 days' storage. According to the least differences required for significance, levels of penicillin up to and including 1,000 units did not cause a mathematically significant decrease in livability as compared to the untreated controls, but the levels of 1,250, 1,500, and 2,000 units brought about a highly significant decline in spermatozoan activity.

Effect of penicillin upon the glycolysis of spermatozoa. The studies of Knodt and Salisbury (4) indicated that the percentage recovery of glucose

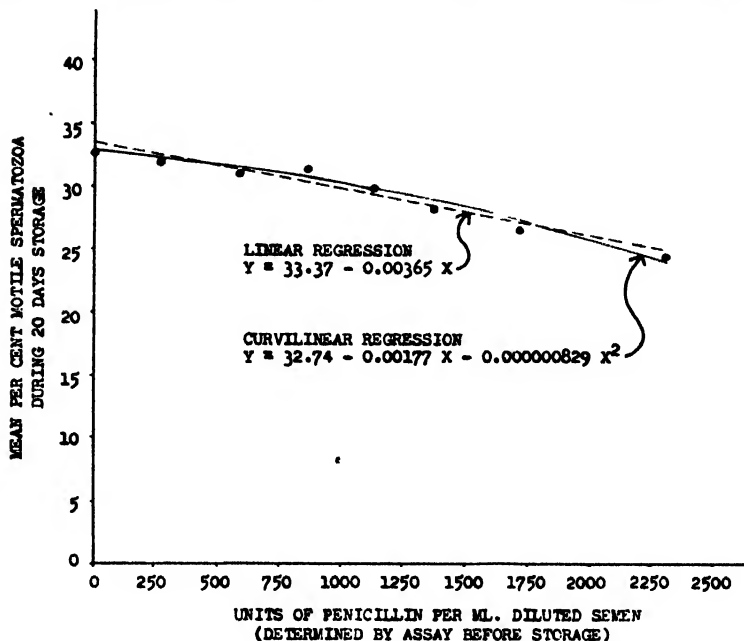


FIG. 1. Relationship of per cent motile spermatozoa during 20 days' storage to level of penicillin, as shown by regression.

as lactic acid was relatively small in diluted bull semen containing no sulfanilamide, but increased when the sulfanilamide was added. In the present study, glucose and lactic acid determinations were made on nine samples of diluted semen stored for 0, 8, and 16 days. Glucose analyses were made according to the method of Somogyi (9), while lactic acid was measured by the method of Barker and Summerson (2). The data are shown in table 2. Analysis of variance involving 216 glucose determinations demonstrated that penicillin significantly reduced the utilization of glucose at all levels. On the other hand, an analysis of the corresponding lactic acid determinations showed no statistically significant differences between the controls and

the various levels of penicillin. The addition of penicillin increased the percentage of glucose loss recovered as lactic acid as compared to the tubes without penicillin. Thus, bacteria may have utilized part of the glucose not recovered as lactic acid in the diluted semen which was not treated with penicillin.

Effect of penicillin upon the bacterial content of diluted semen. Bacterial plate counts were made on five samples of diluted semen using veal infusion agar containing 2 per cent sterile defibrinated ox blood. The samples were plated at dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 with incubation at 37° C. for 72 to 96 hours. Organisms of the coliform group were determined by counts on desoxycholate agar plates which were examined after 24 hours at 37° C. and, if no colonies were present, after an additional 24 to 48 hours. Bacterial counts were made not only on diluted semen after 0, 8, and 16 days of storage, but also on samples of undiluted semen and yolk-citrate diluter stored for the same intervals.

TABLE 2

The effect of penicillin upon the glycolysis of diluted bull semen stored for 16 days at 4.5° C. (mean of 9 determinations)

	Units of penicillin per ml. of diluted semen							
	0	250	500	750	1000	1250	1500	2000
Mg. % glucose* loss	63	46	58	51	43	52	49	44
Mg. % lactic acid gain	46	47	47	49	48	49	46	42
% glucose utilized recovered as lactic acid	73	102	81	96	112	94	94	95

* Reducing substances expressed as glucose.

In the five semen samples studied, no growth of typical coliform colonies on the desoxycholate agar plates was noted.

The data presented in table 3 indicate that penicillin retarded bacterial growth in freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was observed in the tubes without penicillin. The majority of the plates made from both penicillin-treated and untreated semen stored for 16 days possessed a heavy growth of minute colonies which made counting impossible. In four out of 20 possible cases, countable plates were obtained at the 250 and 500 unit levels from the tubes stored for 0 and 8 days. Possibly the raw semen contained bacteria which were relatively resistant to penicillin at these lower concentrations but which were inhibited by the higher concentrations.

The three irregular plate counts obtained in sample numbers 13 and 14 at levels of 1,000, 1,500, and 2,000 units (after storage for 8 days), as well as the heavy growth of pin-point colonies noted at 16 days (table 3), may

TABLE 3
The effect of penicillin upon bacterial growth in diluted semen stored for 8 and 16 days at 4.5° C. (6 ejaculates)

Bull	Sample no.	Bacteria per ml. (in thousands)									
		Undiluted semen	Yolk-citrate diluter	Units of penicillin per ml. of diluted semen							
				0	250	500	750	1000	1250	1500	2000
Before storage											
B	11	0.2	*	*	*	*	*	*	*	*	*
C	12	7.7	*	0.6	*	*	*	*	*	*	*
B	13	1.6	*	0.7	*	*	*	*	*	*	*
A	14	960	*	76	41	*	*	*	*	*	*
B	15	850	*	50	54	25	*	*	*	*	*
After storage for 8 days											
B	11	0.5	*	*	*	*	*	*	*	*	*
C	12	4.5	*	225	*	*	*	*	*	*	*
B	13	0.6	*	1.7	*	*	*	40	*	*	0.8
A	14	200	0.3	75	39	*	*	*	*	6.8	*
B	15	340	*	15	*	*	*	*	*	*	*
After storage for 16 days											
B	11	0.6	78	†	†	†	†	†	†	†	†
C	12	3	*	10	*	*	*	*	*	*	*
B	13	0.5	†	†	†	†	†	†	†	†	†
A	14	1600	*	†	†	†	†	†	†	†	†
B	15	2500	*	15	†	†	†	†	†	†	†

* = < 300 bacteria per ml.

† = > 3,000,000 bacteria per ml. (pin-point colonies too numerous to count).

be due to bacterial contamination of the samples during storage. That is, the tubes from which the subsamples for plate counts were taken were opened every 2 days during storage in order to make motility observations on the spermatozoa. Thus, it is possible that penicillin-resistant organisms were added to the samples. The bacterial counts on the yolk-citrate diluter stored for 8 days were the same, with only one exception, as those obtained prior to storage. These tubes were not opened during the 8-day storage period and thus contamination apparently was avoided.

Total plate counts on portions of freshly collected semen showed considerable variation, ranging from 200 to 960,000 bacteria per ml. with a mean of 364,000 bacteria per ml. for the five ejaculates studied. The semen samples were collected by means of an artificial vagina from three bulls used

TABLE 4

The stability of penicillin in diluted semen stored at 4.5° C. (mean of 9 determinations)

Theoretical units of penicillin*	Units of penicillin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
250	266	270	233
500	592	568	468
750	857	815	719
1000	1133	1152	963
1250	1370	1313	1109
1500	1707	1718	1393
2000	2318	2156	1952
Diluter alone	0	0	0

* Number of units expected based on the total units in the ampules according to the producer.

for natural breeding in the College herd and no special precautions were taken to clean the bulls prior to collection.

Stability of penicillin in diluted semen. Penicillin assays were made on nine samples of diluted semen after storage for 0, 8, and 16 days, not only to determine the stability of the antibiotic but also to obtain more definite information on the actual number of units of penicillin added to the tubes of diluted semen. The latter was of particular interest since producers of penicillin usually add some excess units of penicillin to their ampules. Assays were made according to the standard cylinder plate method of Schmidt and Moyer (7). *Staphylococcus aureus* was the test organism employed. Plain yolk-citrate diluter was also assayed against this organism in order to ascertain whether or not it had any antibacterial activity.

The data in table 4 show that there was practically no loss of penicillin activity in diluted semen stored for 8 days at 4.5° C. Even after storage

for 16 days only a slight decrease in concentration occurred. The temperature employed, as well as the fact that apparently no coliform organisms were present in the samples, was undoubtedly responsible to a large extent for the results obtained. Organisms of the coliform group, particularly *Escherichia coli* (1), are capable of producing a penicillinase which destroys penicillin.

As indicated in table 4 the units of penicillin obtained by assay on portions of the diluted semen immediately following preparation always exceeded the theoretical number of units. Assays on yolk-citrate diluter showed that it possessed no antibiotic activity.

SUMMARY

1. The addition of 250, 500, and 750 Oxford units of penicillin per ml. of diluted semen did not significantly reduce the ability of spermatozoa to maintain motility during a storage period of 20 days. Levels of penicillin ranging from 1,000 to 2,000 units per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a 20-day storage period.

2. In routine artificial breeding, semen is seldom used after holding more than 6 days. When compared with untreated control samples, no significant decrease in maintenance of spermatozoan motility during a 6-day storage period occurred as the result of addition of 250, 500, 750, or 1,000 units of penicillin per ml. of diluted semen, but higher levels of penicillin were deleterious.

3. Within the limits of laboratory experiment, the relation between spermatozoan livability and level of penicillin is well represented by a straight line, the mean percentage of motile spermatozoa during storage for 20 days decreasing by 0.9 per cent for each additional 250 units of penicillin.

4. Penicillin depressed the utilization of glucose at all levels studied (250 to 2,000 units per ml. of diluted semen), while the amounts of lactic acid which accumulated were not significantly affected. Addition of penicillin increased the percentage of glucose utilized which was recovered as lactic acid.

5. Penicillin retarded bacterial growth at all levels in both freshly diluted semen and diluted semen stored for 8 days, whereas considerable growth was found in the semen without penicillin. The initial plate counts for the five ejaculates studied ranged from 200 to 960,000 bacteria per ml. of undiluted semen, with a mean of 364,000 bacteria per ml.

6. There was no appreciable loss in penicillin activity in diluted semen stored for 8 days and only a slight decrease in concentration after storage for 16 days at 4.5° C.

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THE EFFECT OF CONDITIONS OF STORAGE ON THE VISCOSITY OF SWEETENED CONDENSED MILK

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The viscosity of sweetened condensed milk increases with age until a gel structure develops. The time required for the milk to change from a fluid to a gel state depends on the quality of the raw milk, the manufacturing processes employed, the composition of the finished milk, and the conditions of storage to which the product is subjected. This paper is concerned with the effect on viscosity of variations in storage conditions, especially the time and temperature of storage.

Sweetened condensed milk should be smooth and free-flowing, but viscous enough to prevent settling of lactose and rise of fat during storage. When age thickening proceeds to the stage of gelation, the milk is no longer suitable for many food uses. Deterioration in flavor generally accompanies the change in viscosity. The data in this report indicate some of the storage conditions that retard undesirable increases in viscosity.

EXPERIMENTAL PROCEDURE

The preparation of laboratory samples of sweetened condensed milk that were uniform in viscosity from batch to batch and from day to day was found to be very difficult. After extensive trials a technique was developed for the processing of 100 lb. of milk of 3.8 per cent fat and 9.15 per cent solids-not-fat, to which was added during concentration 18 lb. of sugar as a boiled sirup. All temperatures were controlled carefully, and the time required to perform each operation was the same for every batch of milk. The milk was forewarmed in a steam-jacketed kettle and concentrated in a 28-inch vacuum pan equipped with a steam jacket for finishing small batches of milk. Cooling and crystallizing were done in a water-jacketed vessel equipped with a stirrer.

Despite the precautions that were taken in preparing laboratory samples, these age thickened more rapidly than did the commercial milks. The laboratory samples were used chiefly in preliminary tests. The results obtained with the experimental milks differed from those with the commercial products in the magnitude of their viscosities, but the relationship between viscosity and the factors being studied was approximately the same in both types of samples.

The commercial sweetened condensed milk was prepared as parts of regular runs in a large condensery in northern New York. Grade A raw

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milk was used, and all manufacturing operations were conducted according to the best factory practice. The finished product was representative of the highest commercial grade of sweetened condensed milk. The condensed milk was shipped from the plant to the Bureau laboratories by express, the trip requiring 5 or 6 days.

The milks were stored in rooms where the temperature was controlled so that fluctuations did not exceed $\pm 2^\circ$ F. Samples were not moved or disturbed during storage.

Reliable viscosity determinations on sweetened condensed milk are difficult to make. Stebnitz and Sommer (6) constructed a special viscosimeter utilizing the falling-sphere method. The measurements reported here were made under carefully controlled conditions with a McMichael viscosimeter and standardized wires. The determinations were made at 86° F. in a room maintained at this temperature. Each determination was made with a new sample of milk transferred in the same way and without stirring to the viscosimeter cup. Measurements often were made on duplicate samples and sometimes five or six samples were used to establish a single value. All milk samples either had an initial viscosity or soon developed a viscosity high enough to prevent lactose and fat separation during storage. It is believed that the viscosity values obtained during the work reflect the body condition the consumer would find in the milk. The viscosity is reported in poises but, because of the nature of the material, the values are relative rather than absolute.

Consideration was given to the possibility of bacterial growth during storage of the sweetened condensed milk. Rice and Downs (4) found that most of the organisms that might cause age thickening grew when the sugar ratio was less than 62.5 $\left(\text{sugar ratio} = \frac{\% \text{ sugar}}{\% \text{ sugar} + \% \text{ water}} \times 100 \right)$ but that growth sometimes occurred up to 64.5. The increase in titratable acidity found by Rice and Downs to accompany bacterial growth was 0.2 to 0.6 per cent after about 30 to 60 days of incubation.

The sugar ratio of the milks used in these experiments was 62.5 to 63.0. The titratable acidity of the samples stored at various temperatures was not always determined, but the tests that were made showed that the acidity of the milks increased about 0.1 per cent during a 3- or 4-month period at the higher temperatures. The age thickening observed in the sweetened condensed milks discussed in this paper was not considered to be influenced significantly by bacterial changes.

RESULTS

Cooling of the product and crystallization of the lactose in sweetened condensed milk may require 2 or 3 hours at temperatures that affect the viscosity of the milk. The effect of the rate of cooling sweetened condensed milk on

its age thickening is shown in table 1. The data represent the average values from five experiments with five different batches of milk. After the concentrates were dropped from the pan, they were cooled to 86° F. in 10 to 15 minutes and seeded. The cooling process of the rapidly cooled samples was continued, but the slowly cooled concentrates were held and stirred at 86° F. for about 2 hours before cooling was continued. The results indicate that the rate of age thickening is substantially the same for both methods of cooling. The lactose crystals in the rapidly cooled milk were a little larger (about 16 μ) in size than the lactose crystals in the slowly cooled milk (about 12 μ).

The relationship between container size and the age thickening of sweetened condensed milk was investigated. The experiments were done on fresh, commercially manufactured samples that were received in the Bureau

TABLE 1

*The effect of rate of cooling on age thickening of sweetened condensed milk**

Storage time at 86° F. (days)	Viscosity after storage	
	Cooled in 162 min. to 63° F. (poises)	Cooled in 38 min. to 52° F. (poises)
1	115	110
4	204	193
12	365	362
24	583	550

* The data represent average values from 2 skim and 3 whole sweetened condensed milks. The samples were made in the Research Laboratory pilot plant during March and April. After condensation, each batch was divided into two parts, one for slow and the other for rapid cooling. The cooled milk was canned and placed at once in a storage room at 86° F.

laboratories in 30-gallon tight oak barrels. Smaller containers in the form of cans of various sizes were filled with sweetened condensed milk. About 3 gallons of milk was taken from the test barrels for this purpose. During the storage period one of the small cans of milk was opened for each viscosity determination.

The milk in the barrels was sampled through the bungs by means of a 1-inch diameter metal tube inserted diagonally from the bung toward one end of the barrel. Care was taken to close the bungs tightly after sampling. Table 2 shows some results of the study of the effect of container size on the viscosity of sweetened condensed milk. The tight oak barrels of sweetened condensed milk were held on the bilge with bungs down.

Two barrels of the milk received in September, 1944, were put in storage at 86° F. Samples were obtained from one barrel while the other was held unopened until the end of the test. When the sealed barrel was opened at the bung after 76 days, there was insufficient oxygen in the headspace to

support a match flame. The average viscosity of the milk (380 poises) and the titratable acidity (0.55 per cent) were substantially the same as those of the milk in the barrel that had been used for sampling (table 2).

The viscosity of the milk in the barrel that was not opened for sampling was different in various parts of the barrel, after 76 days at 86° F., being 517 poises at the surface and 478 poises at the bottom. In several places near the center of the barrel the viscosity of the milk was 352 poises. When the barrel was filled by the manufacturer, the temperature of the milk was about 50° F., and the milk in the center of the barrel probably required appreciable time to warm to 86° F. In addition, some surface thickening

TABLE 2

Effect of container size on the viscosity of two lots of sweetened condensed milk of commercial manufacture

Storage time	Barrel (30 gal.)	No. 3 can	No. 1 can	Baby can
(days)	(poises)	(poises)	(poises)	(poises)
Milk of September 27, 1943*—storage temperature 70° F.				
1	69	.	69	69
22 " " " " "	70	.	78	81
40 " " " " "	92	.	111	115
92 " " " " "	140	.	.	182
180 " " " " "	148	.	233	255
256 " " " " "	246	.	285	346
357 " " " " "	368	.	446	550
Milk of September 26, 1944†—storage temperature 86° F.				
1	39	39	39	39
8	64	88	86	86
17	80	101	108	119
35	143	168	176	176
74	385	407	429	429

Composition figures furnished by the manufacturer:

* 9.25% fat, 23.0% M.S.N.F., 42.75% sucrose, 25% water.

† 8.73% fat, 23.62% M.S.N.F., 42.75% sucrose, 24.9% water.

Sugar ratio of these milks = 63.

may have been caused when this barrel dried and leaked slightly between the staves. This occurred in the middle of the storage period but the staves tightened up in 3 days when the humidity in the storage room was raised.

The effect of time and temperature of storage upon the viscosity of sweetened condensed milk was investigated. Samples were prepared June 1, 1945, by a cooperating manufacturer as part of a large commercial batch. The samples contained 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent moisture. A barrel of this sweetened condensed milk was shipped to a canning plant, where it was re-packed in special 2-ounce cans on June 8. Four hundred of these small cans were received in these laboratories June 21 and stored at six different tempera-

tures. Each can held enough milk to make one viscosity determination. Some of the results are plotted in figure 1.

This milk showed a large increase in acidity with age, but apparently this was not caused by bacterial growth. The figures determined on the manufacturer's milk in six of the 2-ounce cans showed an average standard plate count of 4,380 and a titratable acidity of 0.43 per cent. After 640

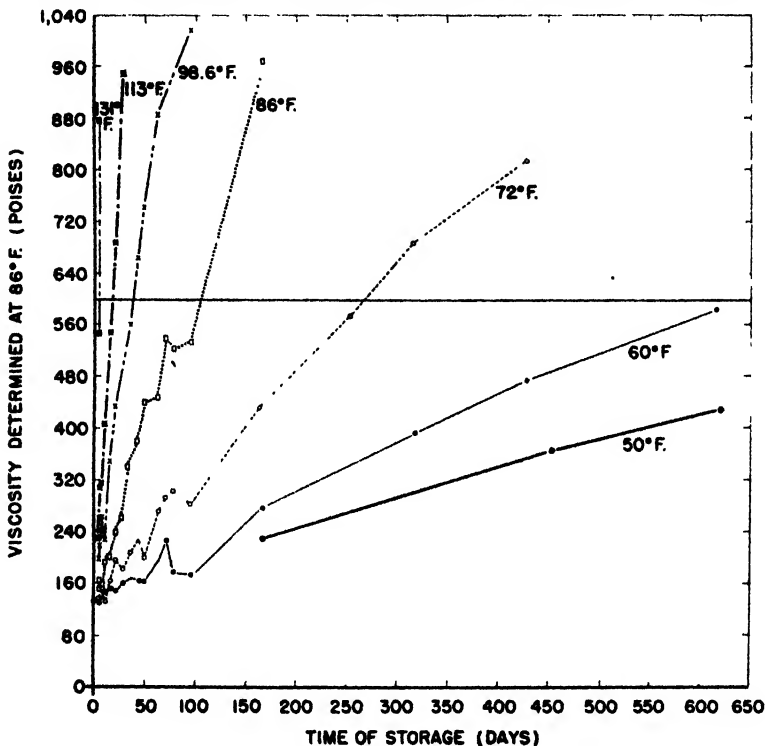


FIG. 1. The effect of time and temperature of storage on the viscosity of sweetened condensed milk stored in 2-ounce cans. All samples were from the same batch of milk, but the samples used for storage at 50° F. were delayed 2 weeks in transit at summer temperatures, causing an undue initial increase in viscosity. The composition of the milk was 9.61 per cent fat, 23.91 per cent M.S.N.F., 41.65 per cent sugar, and 24.83 per cent water.

days of storage at different temperatures, the titratable acidity and the acid intensity of this milk were: 50° F. storage, 0.84 per cent and pH 6.01; 60° F. storage, 0.85 per cent and pH 6.09; 72° F. storage, 1.0 per cent and pH 5.90. No signs of bacterial growth were found in samples held at these temperatures for 640 days.¹ Anaerobe tubes, direct smears, and standard

¹ Bacteriological examination of this milk was made by Harold R. Curran of these laboratories.

plates were practically negative. While it was not shown that growth of organisms was absent during the whole storage period, the available evidence indicates that the acidity increase was due to chemical changes rather than to bacterial activity.

The storage temperature of a group of the 2-ounce cans of sweetened condensed milk was varied from 60° F. to 98° F. by shifting the cans every 24 hours from one temperature to the other. The initial viscosity of the milk was 132 poises, but after storage for 170 days a soft gel had formed with a viscosity of 1,155 poises. By reference to figure 1 it may be determined that this is the viscosity the milk would have reached if it had been held 170 days at a constant temperature of 89° F.

Sweetened condensed milks that were stored for several months at 0° F. did not show important physical changes. There was no measurable change in viscosity and no apparent change in the dispersion of the protein or the fat. There was no protein flaking or insolubility, such as occurs in frozen unsweetened milk. There was an increase in the size of lactose crystals in unsweetened condensed milks held at 0° F. only when the lactose was incompletely crystallized as a result of improper cooling during the manufacturing process.

DISCUSSION

The keeping quality of sweetened condensed milk is closely associated with its viscosity. Whenever sweetened condensed milk age thickened to a viscosity of 600 to 800 poises, it generally was no longer suitable for use in high-grade food products. Off flavors often developed and the milk was too viscous to handle easily. A soft gel structure was present at 1,000 poises. The gel could be reduced by stirring, but it re-formed when the milk remained undisturbed.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under the same conditions of temperature.

The data of table 2 indicate that sweetened condensed milk packed in barrels will remain fluid a little longer than milk packed in cans when both are held under the same conditions of storage. The temperature of milk in cans follows fluctuations in storage temperature more closely than does the temperature of milk in barrels. Commercial experience indicates that the sweetened condensed milk in the middle of a 50-gallon barrel will require about 7 days to reach 85° F. after previous storage at about 60° F. (1). A longer time is required for the reverse change to take place. Cool milk packed in barrels will remain cool and thin longer after the container is placed at a high temperature than will milk in small cans.

These considerations, and the fact that high sugar concentrations stabilize the milk and retard viscosity changes, support the suggestion (2)

that bulk sweetened condensed milk may have a minimum sugar ratio of 60, while the sugar ratio of the canned milk should be at least 62.5. However, a bulk sweetened condensed milk with a sugar ratio of 62.5 will be superior to one that contains less sugar.

Data from the curves of figure 1 were used to prepare figure 2, in which the logarithms of the viscosities are plotted against the temperatures of storage.

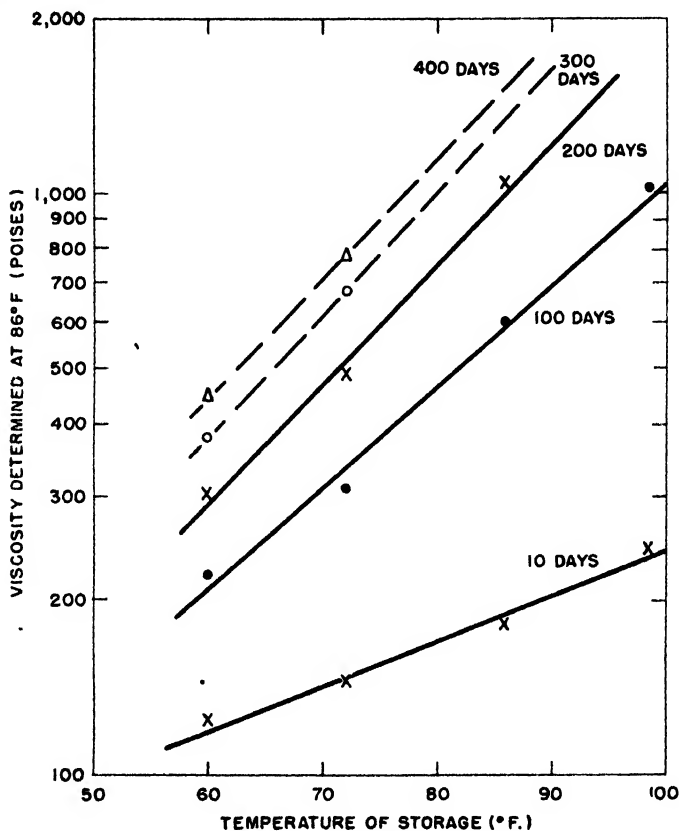


FIG. 2. The effect of time and temperature of storage on the viscosity of sweetened condensed milk. Data taken from figure 1.

The time required for the sweetened condensed milk to reach a viscosity of 600 poises at different temperatures is indicated in figure 1. When these temperature data were plotted against the logarithms of the time of storage, straight line no. 1, figure 3, was obtained. Values for 30, 40, and 50° F., obtained by extrapolation of curve no. 1, figure 3, are 4,950, 2,460, and 1,225 days, respectively. Other data also are plotted on figure 3.

If n = viscosity and c, c', c'' = constants, the relationship shown in figure 1 may be expressed as follows: $n = c'$ (time of storage) or $\log n = c''$ (log time), while for figure 2 $\log n = c$ (temperature of storage); then c (temperature) = c'' (log time), the relationship shown in figure 3 where the viscosity is constant.

The data given in figure 1 and re-plotted in figures 2 and 3 show that

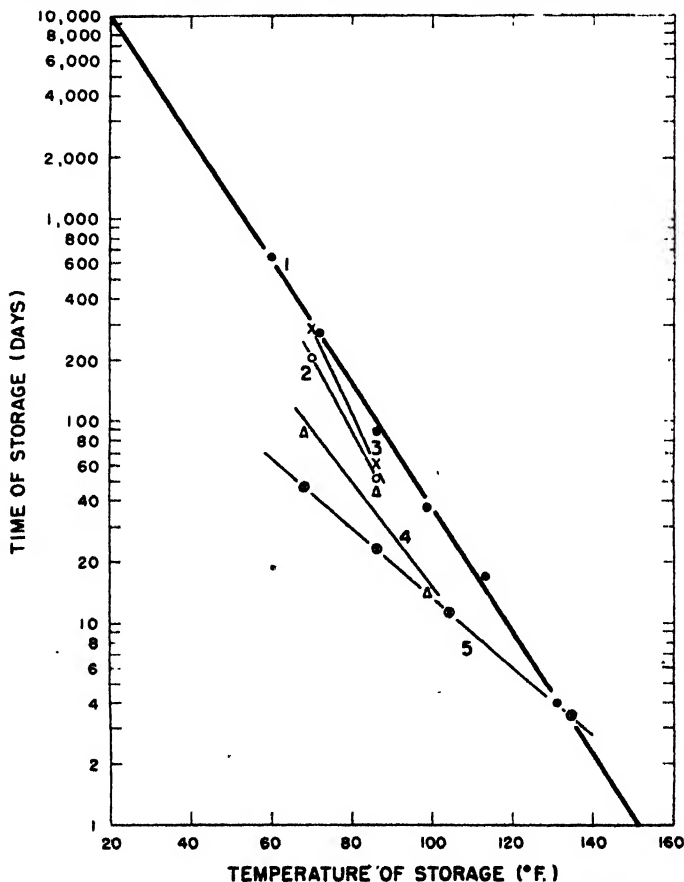


FIG. 3. The relationship between the time and temperature of storage in the development of viscosity of sweetened condensed milk: Curve 1, data from figure 1, viscosity of 600 poises. Curve 2, data from table 2, viscosity of 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in baby-size cans. Curve 3, data from table 2, viscosity equals 300 poises. The points for 70° F. and for 86° F. were obtained with different milks stored in barrels. Curve 4, data from Rogers *et al.* (5), viscosity of 12° rotation on special viscosimeter. Curve 5, data from Leighton and Mudge (3) in which the time is given in hours (not days) required for the milk to start thickening.

the viscosity of sweetened condensed milk increases arithmetically with time of storage but logarithmically with temperature of storage. For conditions of constant viscosity the time of storage varies logarithmically with the temperature of storage.

Although the logarithms of the storage times required for a sweetened condensed milk to reach a certain viscosity, plotted against the temperatures of storage, yield a straight line (fig. 3), the slope of this line may vary with different milks. The milk used by Leighton and Mudge (3), figure 3, gave a line of different slope from that of the other milks. The data of Leighton and Mudge were obtained by observing the number of hours required for the milk to start thickening. It seems probable that most commercial milks will thicken much as did milk no. 1 of figure 3. It should be possible to estimate the length of time a sweetened condensed milk will retain a satisfactory viscosity at various storage temperatures by using the data of figure 3. The rate of thickening approximately doubles with each increase of 10° F. between 30 and 60° F.

In some cases the slope of the time-temperature curve of a milk may differ from that of curve 1, figure 3. A line may be drawn for any milk if two points are obtained. To secure these points quickly, about six samples of a milk may be held at each of two high temperatures such as 98° and 120° F. Viscosity determinations should be made at 1- or 2-day intervals and curves drawn like those of figure 1. Two points having the same viscosity value then may be used to construct a time-temperature curve patterned after that of figure 3. Other points may be taken from the curve or they may be calculated from the geometric equation for a straight line. The equation may be stated in the following form:

$$\frac{\log y - \log y_1}{\log y_2 - \log y_1} = \frac{x - x_1}{x_2 - x_1}$$

when
 y = storage time
 x = storage temperature

y_1 and y_2 = storage times at x_1 and x_2 storage temperatures. For a milk similar to that shown in curve 1, figure 3, the storage time (y) required to reach a viscosity of 600 poises at a given temperature (x) may be found from the equation calculated from the plot:

$$\log y = 4.64 + \frac{2}{39} x.$$

It is important to note that the time-temperature relationship of figure 3 will hold only when the storage temperature is constant. If the storage temperature fluctuates, the changes in viscosity will be dependent upon the extent of the changes.

The theoretical freezing point of sweetened condensed milk is about 5° F., but only a few ice crystals form at this temperature. Since all the

moisture in sweetened condensed milk will freeze only at a much lower temperature, neither the milk nor the container is damaged by storage at temperatures considerably below 0° F. Sweetened condensed milk that was held at 0° F. for many months did not change measurably in viscosity. Extrapolation of Curve 1, figure 3, indicates that 24,000 days (65 years) at 0° F. would be required for this milk to reach a viscosity of 600 poises.

SUMMARY

The viscosities of sweetened condensed milks held in the same storage room increased a little more rapidly in the milks packed in small cans than they did in the milks packed in barrels.

The viscosity of sweetened condensed milk increased logarithmically with increases in storage temperature and arithmetically with increases in storage time. For conditions of constant viscosity, time varied logarithmically with temperature. Viscosity values may be predicted by applying this relationship to time-temperature data from high temperature, accelerated storage tests.

The viscosity of sweetened condensed milk increased at about the same rate during the cooling and crystallization periods as it did during storage under identical conditions of time and temperature.

The authors wish to express their appreciation to P. J. Haymes, H. D. Wilder, and A. R. Davis, of the United Milk Products Company, for their numerous suggestions and their active interest in this project and for their manufacture of the commercial samples of sweetened condensed milk.

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DETERMINATION OF CHEESE LIPASE¹

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In recent years lipolysis has been directly associated with flavor development in Cheddar cheese. In order to study directly the fat breakdown in Cheddar cheese, procedures for the quantitative determination of the lipolytic agents responsible are necessary.

A method for the determination of milk lipase previously was reported by the present authors (1). Since no methods for the quantitative determination of cheese lipase have been described, it was necessary to devise one. In the present paper such a method is presented. It is similar in principle to the milk lipase determination previously reported (1) and, similarly, has the limitation that it measures only enzymes capable of hydrolyzing tributyrin.

EXPERIMENTAL

Effect of pH on cheese lipase activity. In figure 1 the pH activity curve for the hydrolysis of tributyrin by cheese extracts is given. Determinations of enzyme activity at various pH values were carried out according to the procedure subsequently outlined under "Quantitative Determination of Cheese Lipase" with the following exception: In place of 2 ml. of 0.38 molar aniline buffer, 2 ml. of a 0.38 molar aniline, 0.38 molar phosphate buffer were added. By means of this composite buffer, pH was controlled throughout the pH range desired.

It will be seen from the figure that tributyrin is split most rapidly at pH 5. There also is a secondary optimum at pH 6.5 to 7.0. This secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the lipolytic activity seems quite constant, however, throughout the cheese-ripening period. The presence of two peaks in the pH optimum curve of cheese lipase indicates strongly that two or more lipases are present. The enzymes active at pH 6.5, however, are not active in ripening cheese, since the pH of the cheese seldom rises above 5.4. Therefore, only the lipolytic activity at pH 5 was considered important.

Effect of buffers on cheese lipase activity. In the development of a method for the estimation of cheese lipase, it was necessary to find a buffer which did not affect the lipase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese lipase. As may be seen, the citrate, acetate, and

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phthalate buffers had an inhibitory effect on the lipase. None of the other three buffers inhibited the enzyme. Any buffer containing the carboxyl group seems to inhibit the lipase. The flattening of the curves in figures 3 and 4 when a titration greater than 0.40 ml. of 0.1 N NaOH is obtained per 5 ml. of incubation mixture probably is due to reversible combination of the butyric acid with the enzyme to form an enzyme-product complex. The buffers containing carboxyl groups probably inhibit the lipase in the same way. The phosphate buffer was not used in the cheese lipase method as

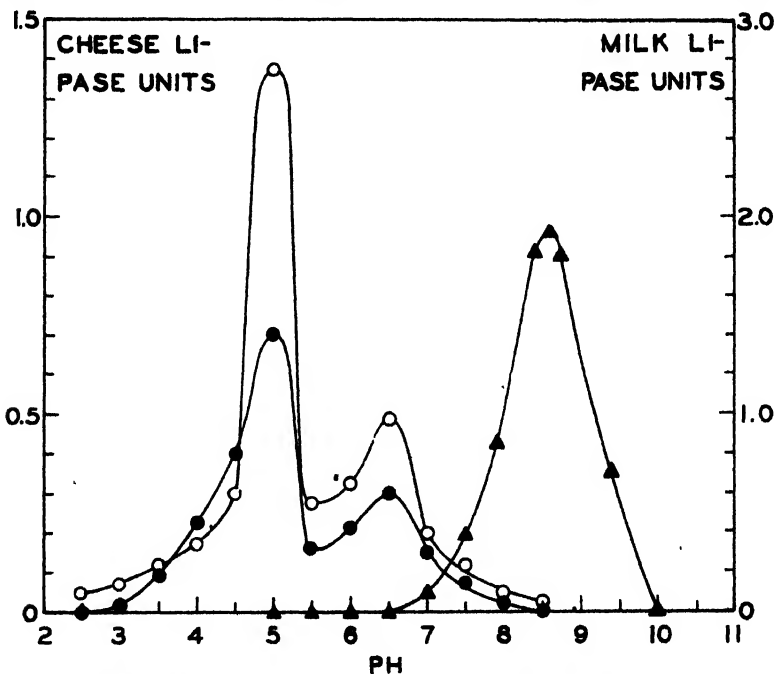


FIG. 1. Effect of pH on rate of tributyrin hydrolysis by cheese extracts. The effect of pH on the rate of tributyrin hydrolysis by milk lipase is presented for comparison. Triangles represent milk lipase, dots represent 2-month-old raw milk cheese, and circles represent 10-month-old raw milk cheese.

finally adopted because it has very little buffering capacity at pH 5. Both the pyridine and aniline buffers have good buffering capacity at pH 5. Since the aniline buffer was the more convenient to use, it was chosen for use in the final method.

Effect of activators on cheese lipase activity. Various compounds known to possess definite activating properties for other enzymes were tested for activating effect on cheese lipase. As may be seen in table 1, none of them gave activation; most of them were inhibitors.

TABLE 1

The effect of activators and buffers on cheese lipase activity

Substance used	Concentration	Apparent cheese lipase in sample
	Activators	(units/ml.)
None		0.88
Zinc chloride	0.001	0.82
Potassium cyanide	0.001	0.64
Manganous sulphate	0.001	0.80
Magnesium chloride	0.001	0.88
Calcium chloride	0.001	0.90
Cysteine	0.001	0.78
	Buffers	
Aniline	0.0625	0.70*
Pyridine	0.1	0.70
Phosphate	0.1	0.70
Citrate	0.1	0.16
Phthalate	0.1	0.22
Acetate	0.1	0.34

* The lipase solution used in the buffer experiments was not the same as that used in the activator experiments.

Variation of hydrolysis rate with amount of tributyrin present. Figure 2 shows that the rate of hydrolysis of tributyrin by cheese lipase is affected by concentrations of tributyrin up to 1.5 per cent. At this level and above, the hydrolysis rate appears to be constant.

Kinetics of cheese lipase. In figure 3 the relation between quantity of cheese lipase and tributyrin hydrolysis is presented. It may be seen that the tributyrin hydrolysis obtained is directly proportional to the quantity of cheese lipase present, up to the point when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

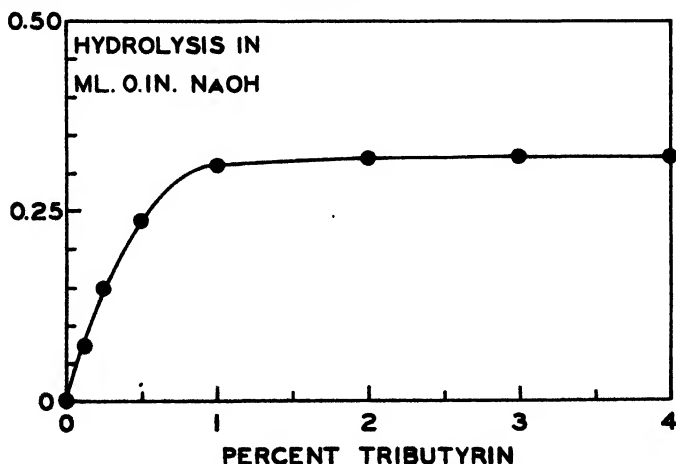


FIG. 2. Variation of hydrolysis rate with amount of tributyrin present.

In figure 4 the effect of incubation time on tributyrin hydrolysis by cheese lipase is shown. It may be seen that the hydrolysis of tributyrin by cheese lipase at pH 5 is linear up to the time when 0.40 ml. of 0.1 N butyric acid is present in 5 ml. of incubation mixture.

The enzyme unit used in the cheese lipase determination is based on the relationships presented in figure 3.

QUANTITATIVE DETERMINATION OF CHEESE LIPASE

In the method finally adopted, the following procedure is used. Twenty grams of the cheese sample to be tested are weighed out with an accuracy of ± 0.1 g. To the weighed sample in a Waring Blender or other suitable

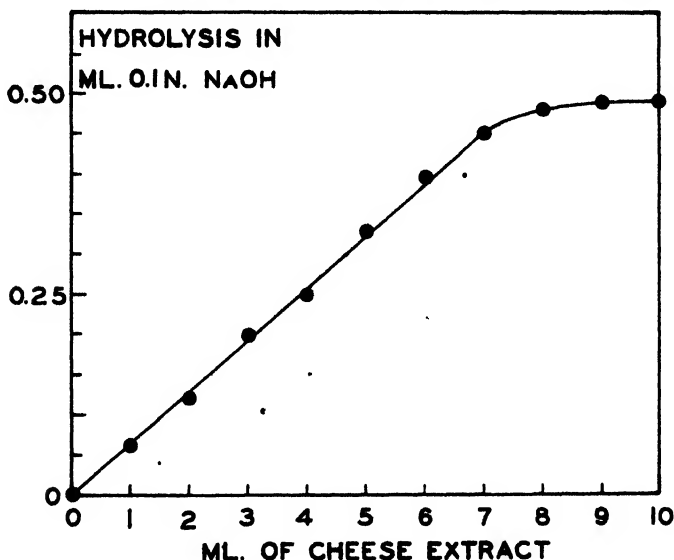


FIG. 3. Relation between quantity of cheese lipase and amount of tributyrin hydrolysis.

mixer, enough distilled water is added to make a total volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set to high speed and allowed to run 7 more minutes. At the end of this time the mixture is in the form of a smooth, white suspension. This suspension is transferred quantitatively to a small hand homogenizer using a minimum amount of distilled water. The suspension then is run five to six times through the homogenizer, which further insures complete extraction of the enzyme. After quantitative transfer to a 250-ml. centrifuge tube, the suspension is centrifuged for 10 minutes at 2,000 r.p.m. At the end of this period the solid material in the suspension will have settled out, leaving a faintly turbid supernatant which is drawn off. The residue is washed care-

fully and centrifuged at 2,000 r.p.m. for 10 minutes with three successive 20 ml. portions of distilled water, which all subsequently are added to the turbid supernatant. The supernatant plus the three washings is diluted to a total volume of 200 ml. and the resulting cheese extract is used directly as the enzyme sample to be analyzed. Experiments have shown that all the lipolytic activity present in the cheese is washed free from the cheese curd by the above procedure.

For the analysis, a suitable volume (depending upon its activity) of the

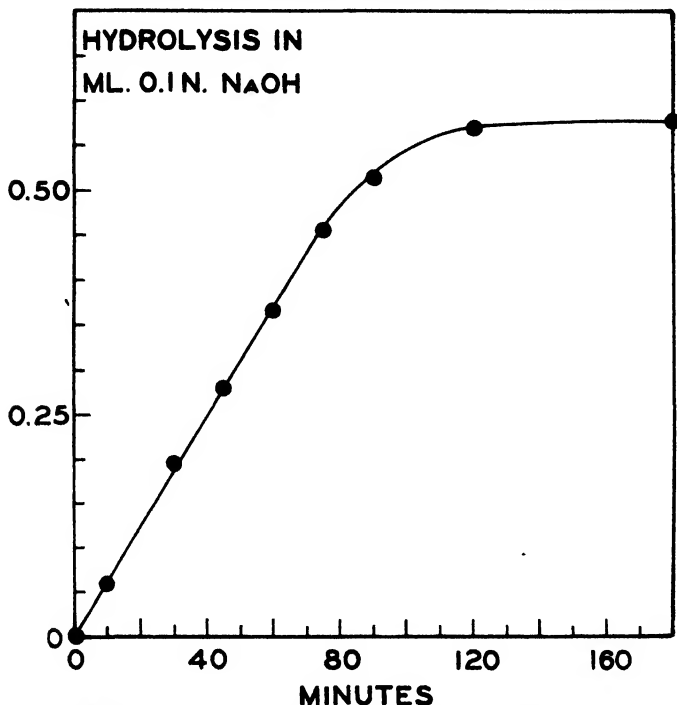


FIG. 4. Effect of incubation time on tributyrin hydrolysis by cheese lipase.

cheese extract is added to 2 ml. of 0.38 molar aniline buffer at pH 5. This solution is diluted to a volume of 12 ml. with distilled water and placed in a water bath held at 40° C. After the solution has attained this temperature, 0.2 ml. of tributyrin is added, making a total volume of 12.2 ml. The mixture is shaken vigorously for 1 minute, and a 5-ml. aliquot immediately is removed for titration. After an incubation period of 60 minutes² at 40° C., the tube and its contents are shaken for 30 seconds and another 5-ml. aliquot is titrated. The difference in titration between the two aliquots

² In all the experimental work presented, incubation times of 60 minutes were used unless otherwise specified.

represents tributyrin hydrolysis. The titration is carried out as follows: The 5-ml. aliquot is pipetted into a 50-ml. Erlenmeyer flask containing 5 ml. of a 0.02 per cent solution of thymolphthalein in 95 per cent alcohol. After addition of 2 ml. of ether, the solution is titrated with 0.1 N alcoholic NaOH from a burette calibrated at 0.01-ml. intervals. Titration is carried to a definite blue color. It is convenient to prepare an artificial endpoint comparison flask containing a dilute aqueous solution of CuSO_4 and CoCl_2 to which enough alumina cream has been added to give a close resemblance to the actual titration flask.

One cheese lipase unit is defined as the amount of enzyme which, when diluted to 12.2 ml. and incubated for 60 minutes under conditions of the determination as described above, will give a titration increase of 0.1 ml. of 0.1 N NaOH for a 5-ml. aliquot. For instance, if 4 ml. of cheese extract

TABLE 2

Comparison of volatile acid distillate titration differences and direct titration differences

Enzyme sample no.	Amount of hydrolysis of tributyrin by titration differences of volatile acid distillates	Amount of hydrolysis of tributyrin by direct titration differences
	(ml. 0.1 N NaOH)*	(ml. 0.1 N NaOH)*
A-1	0.05	0.05
A-2	0.36	0.34
A-3	0.42	0.43
A-4	0.17	0.19
A-5	0.27	0.27
A-6	0.12	0.11

* All values expressed as ml. 0.1 N NaOH represent the amount of hydrolysis of tributyrin by various dilutions of cheese enzyme sample A.

are used, and the observed titration increase is 0.36 ml. of 0.1 N NaOH, the extract contains 0.9 enzyme unit per ml. The linear relation here assumed between titration increase and amount of enzyme is shown to be justified by figure 3 and table 3.

If the observed titration increase is more than 0.40 ml., the determination should be repeated with a smaller volume of cheese extract. If the titration increase is less than 0.1 ml., a larger volume should be used. Cheese extracts have been found to retain their activity for at least 10 hours in the refrigerator.

RELIABILITY OF THE METHOD

Evidence that the titration used measures only glyceride hydrolysis is afforded by table 2, where direct titration figures are compared with titrations of volatile acid distillates of equivalent aliquots. Good agreement was obtained in all cases. The volatile acid distillates were obtained as follows: A 5-ml. aliquot of the incubation mixture was pipetted into the distillation

The pH immediately was adjusted to 2 (red to thymol blue) with 5 N H_2SO_4 . A constant volume distillation then was carried out until about five times the volume of the original sample had been distilled over. The distillates obtained were titrated to the phenol red endpoint.

The reliability of the method and of the standard curve also was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 3. As may be seen, determinations agreed within 10 per cent.

TABLE 3

Effect of sample size on apparent cheese lipase content of a cheese extract

Sample size	Titration differences	Lipase content
(ml.)	(ml. 0.1 N NaOH)	(units/ml.)
1.0	0.07	0.70
1.0	0.08	0.80
3.0	0.23	0.77
3.0	0.23	0.77
5.0	0.38	0.76
5.0	0.39	0.78
6.0	0.45	0.75
6.0	0.46	0.77
7.0	0.47	0.67

DISCUSSION

It should be emphasized that the method outlined here has a number of failings. Since the lipolytic activity of Cheddar cheese undoubtedly is due to a mixture of lipases produced by the mixed flora of the cheese, any method of quantitative estimation will give consistent results only if the ratio in which the various lipases are present remains unchanged. This is true because various substrates are attacked at different rates by different enzymes. If a mixed substrate such as butterfat is used, the determination does not inform us whether the fatty acids liberated are the highly flavored volatile fatty acids or the relatively tasteless higher fatty acids. On the other hand, if a pure substance is used as a substrate, the determination can measure only activity toward this substrate, and again no direct information is obtained regarding liberation of the particular acids of interest to the investigator.

Any lipase determination will measure only lipases active at the pH of the determination. The present determination will not detect the presence of lipases active only at high pH values, since the determination is designed to measure only those enzymes active at pH 5.

SUMMARY

1. A method is presented for the quantitative determination of lipolytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

2. Hydrolysis of tributyrin by the Cheddar cheese lipase system is most rapid at pH 5. A secondary optimum occurs at pH 6.5 to 7.0.

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LIPASE ACTIVITY DURING MAKING AND RIPENING OF CHEDDAR CHEESE¹

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In recent years it often has been assumed that rancidity in cheese is due to the production of lower fatty acids such as butyric acid by the action of milk lipase on natural milk fats. It first was suggested by Rice and Markley (18) that milk lipase was one of the causes of rancidity in cheese. Lane and Hammer found that: (a) typical rancid Cheddar cheese could be produced experimentally by the addition of sources of lipase, such as rennet paste (11) and pancreatin (13), to cheese milk, and (b) typical rancid Cheddar cheese could be produced experimentally by the addition of homogenized raw cream to raw skim milk as a means of milk lipase activation (12). Hood *et al.* (10) confirmed the reports of Lane and Hammer and further found that typical rancid Cheddar cheese could be produced experimentally under commercial conditions by vigorous agitation of raw cheese milk at various temperatures (7, 8). Since these earlier publications, Hlynka and Hood (2, 3), Hlynka *et al.* (5, 6, 9), and others (1, 14) have reported a great deal of work which has been carried out on the basis of the milk lipase theory. A large part of this work has been carried out with commercial lipases or crude enzyme preparations as the source of experimental lipase. There is no reason to believe, however, that the lipases from these sources have properties comparable to milk lipase. Moreover, commercial lipase preparations and crude enzyme preparations generally contain many other types of enzymes as impurities.

In a brief preliminary report (15) Peterson and Johnson pointed out that milk lipase is inactive at the pH of Cheddar cheese and is completely absent from Cheddar cheese after pressing. The data on which these conclusions were based are presented in this paper. In a recent paper, Hlynka and Hood (4) also concluded that milk lipase is inactive in Cheddar cheese after it is made.

The purpose of the present paper is to present studies of the differences in cheese lipase content during the making and ripening of raw and pasteurized Cheddar cheese from the same milk. In the preliminary report (15) it was mentioned that lipolytic activity of a type different from that of milk lipase gradually appears in ripening Cheddar cheese. This

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lipolytic activity has been termed "cheese lipase" by the present authors (17). Cheese lipase is distinctly different from milk lipase in its pH optimum (17) and its chemical kinetics with relation to the hydrolysis of tributyrin (16, 17).

METHODS

Milk lipase. The method for the determination of milk lipase previously reported by the present authors (16) was used for all milk lipase analyses. In this method, the degree of hydrolysis of tributyrin at 40° C. by milk lipase in a solution buffered at pH 8.5 is used as a measure of milk lipase activity.

Cheese lipase. The method for the determination of cheese lipase reported by the present authors (17) was used for all cheese lipase analyses. In this method the degree of hydrolysis of tributyrin at 40° C. by cheese lipase in a solution buffered at pH 5.0 is used as a measure of cheese lipase activity.

Cheesemaking procedure. The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. Small vats of cheese were made (460 lb. of milk). Each such vat yielded two 22-lb. cheeses. Cheeses were made by an experienced maker according to standard procedures. The green cheeses produced were kept at 68° F. for a period of 2-3 days and then were transferred to a cold room (40-45° F.) for the remainder of the ripening period.

To make both raw and pasteurized milk cheese from the same milk, 920 lb. of mixed, raw, whole milk was divided into two equal portions. The first portion received no treatment. The second portion was pasteurized by holding 30 minutes at 145° F. Each portion then was placed in a separate vat, and both received identical subsequent treatment according to standard Cheddar cheesemaking procedures.

A. ROLE OF MILK LIPASE IN CHEDDAR CHEESE

Loss of milk lipase during making. In table 1 the stability of milk lipase at various temperatures and pH values is presented. From these data it would be expected that under the pH and temperature conditions used in the making and ripening of Cheddar cheese (table 2), there is little possibility of milk lipase being present in green or ripe Cheddar cheese. Assuming it was present, milk lipase is inactive at a pH of 6.5 or lower (16, 17). Therefore, at the pH of ripening Cheddar cheese (5.0-5.4), milk lipase would have no effect even if present.

In order to obtain direct substantiation for the above conclusions, milk lipase estimations were made before, after, and, if necessary, during any operation in the making and ripening of raw milk Cheddar cheese that might affect the milk lipase content. A number of such experiments were

TABLE 1
Stability of milk lipase at various pH values

pH	Per cent of lipolytic activity remaining after 30 min. incubation		
	(41° F.)	(68° F.)	(104° F.)
4.0	58.2	14.4	0
4.5	72.7	41.0	0
5.0	88.1	48.0	8.1
5.5	99.1	72.3	18.2
6.0	99.1	97.1	44.8
6.5	99.2	98.9	92.0

carried out. The results of one representative experiment are presented in table 2.

As may be seen from the table, no significant change in milk lipase content takes place until the rennet is added to the raw milk. A rapid rise in milk lipase content immediately results. The cause of this rise is not definitely known since no lipolytic activity at pH 8.5 is present in rennet

TABLE 2
Role of milk lipase in Cheddar cheesemaking

Time	Temperature	pH	Source of sample	Milk lipase content*
(min.)	(° F.)			
0	55-57	6.60	Raw milk after addition to vat	29.0
13	86	6.60	Raw milk after warming to 30° C.	27.8
18	88	6.59	Raw milk after addition of starter†	27.8
72	88	6.59	Raw milk after addition of rennet‡	41.5
101	88	6.51	Coagulum after cutting of the curd	25.2
110	88		Coagulum before heating	24.0
133	104	6.48	Curd after heating to 104° F.	16.4
188	96	6.29	Curd during firming process	7.63
208	92	6.04	Curd just after dipping	1.36
208	91	6.12	Whey just after dipping	5.11
368	91	5.46	Curd just after milling	0.78
(hr.)				
24	Room temp.	5.31	Cheese just after pressing	0.00
(days)				
5	40-45	5.28	Cheese	0.00
10	40-45	5.27	Cheese	0.00

* Milk lipase content expressed in all cases as the number of milk lipase units per gram of cheese. All figures are based on the amount of cheese obtained, which equaled 9.72 lb. per 100 lb. of milk.

† One per cent inoculum of an 18-hr. commercial starter culture added.

‡ Commercial rennet added in the amount of 10 ml. per 100 lb. milk.

alone. Since this apparent milk lipase activity disappears rapidly, it is readily seen that the factor responsible is quite unstable. There are two important inactivations or removals of true milk lipase activity. The first takes place during the heating of the coagulum to 104° F. and the holding between this temperature and 96° F. for 55 minutes. After this treatment less than one-third of the original milk lipase activity remains. This is due not only to the temperature effects but also to the low pH range encountered (6.0–6.5). The combination of these two adverse factors is more effective in the inactivation of milk lipase than either one alone. Another large loss of milk lipase occurs during the removal of whey. After dipping, less than 5 per cent of the original milk lipase remains in the curd. Only a small amount of milk lipase remains in the cheese curd just after the milling operation. Due to the low pH (5.3–5.5) of the curd, this small amount of milk lipase is rapidly inactivated, and no milk lipase activity can be found in the green cheese 24 hours after the start of making. As may be seen from the table, analyses were also run after 5 and 10 days of ripening. No milk lipase activity was found.

Discussion. There probably are a number of lipases present in milk. It is very probable that the present method of measuring milk lipase activity does not include all of them. It also is probable, however, that those lipases present in milk which are not capable of splitting tributyrin have little or no effect on the production from milk fat of those fatty acids which could contribute to the flavor of Cheddar cheese. The fatty acids contributing to cheese flavor probably include only those having from two to ten carbon atoms. The fatty acids containing more than ten carbon atoms have little or no taste. Since the fatty acids capable of contributing flavor to cheese must lie within this narrow range of possible number of carbon atoms, there is no reason to believe that a lipase exists in milk capable of splitting, for example, caprylic acid but not butyric acid from a glyceride ester.

As far as rancidity in cheese is concerned, there is no doubt that tributyrin is the most logical choice for a substrate in the measurement of milk lipases possibly causing the rancidity. This is true since rancidity in cheese generally is attributed to the production of lower fatty acids such as butyric acid. There is, of course, the remote possibility that a lipase present in milk would release butyric acid from mixed triglycerides and not from simple triglycerides.

B. LIPASE CONTENT OF CHEDDAR CHEESE AFTER MAKING

Lipase content of raw and pasteurized Cheddar cheese. After a ripening period of approximately 5–20 days, lipolytic activity of the cheese lipase type is found in green Cheddar cheese. In figure 1 the averages of the lipolytic activities of six pairs of pasteurized milk cheeses and raw milk

cheeses made by the procedure described under "Methods" are given. The average per cent deviation of the individual lipase analyses from the mean was less than 3 per cent.

The lipolytic activity in raw milk Cheddar cheese will be considered first. In figure 1 it will be noted that no lipolytic activity at pH 5 is present in the milk at the time it is placed in the vat. This is in accordance with data presented previously (16, 17) showing that milk lipase is inactive below pH 6.5. The first increase in lipolytic activity occurs at the time of addition of the rennet, which is about 75 minutes after the start of making. Since there is a similar increase in the lipolytic activity of corresponding

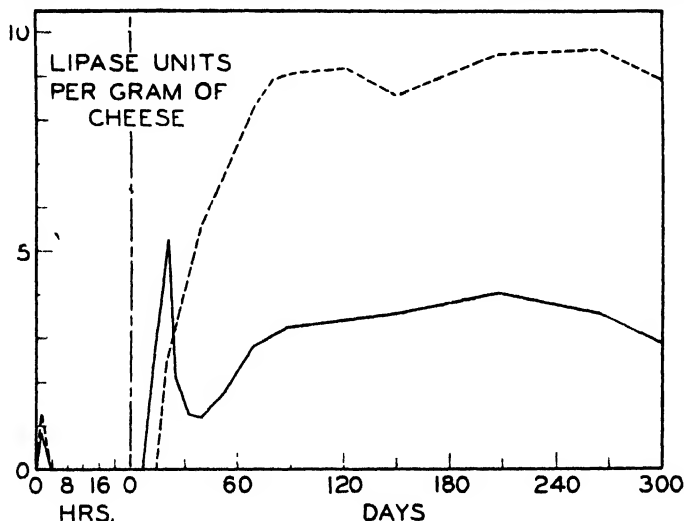


FIG. 1. Lipase activity at pH 5 in Cheddar cheese during making and a 300-day ripening period. Average values for six pairs of cheese. Solid line, pasteurized milk cheeses; broken line, raw milk cheeses.

pasteurized milk cheeses, undoubtedly this effect is caused by lipolytic enzymes which are active at pH 5 and are present in the rennet as impurities. Since this lipolytic activity attributable to rennet disappears at the time of dipping, it can have no effect on the cheese during the ripening period. After dipping there is no lipolytic activity present until after the cheese is in its final form and has been stored 5-20 days. At this time lipolytic activity is detectable, and it increases in amount steadily up to 80-90 days after making. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in large amounts at an earlier stage in the ripening period than in raw milk Cheddar cheese. The significance of this will be discussed later.

Figure 1 shows that the amount of lipolytic activity at pH 5 present during the making of the cheese is very small compared to the lipolytic

activity at pH 5 of the cheese during the ripening period. Therefore, even if this lipolytic activity at pH 5 present during making is carried over into the cheese in a reversibly inactivated form, its role in ripening must be small.

At most a negligible portion of the total lipolytic activity of the cheese is contributed by the milk or rennet during making. Of the possible sources of the lipolytic activity, the bacterial flora of the cheese is the most logical. No lipolytic activity is detectable in the green cheese during the first 5-6 days after making, and most of the cheese lipolytic activity is set free between the fifth and hundredth day of the ripening period after making. Since very few of the lactic acid bacteria present in Cheddar cheese are strongly lipolytic in their normal life cycles, it is suggested that the lipolytic activity of cheese at pH 5 may represent endocellular bacterial lipases of these organisms liberated by bacterial autolysis (19).

The lipolytic activity at pH 5 of pasteurized milk cheese also is presented in figure 1. No important differences in the lipolytic activities of raw and pasteurized milk Cheddar cheese during making are apparent. The first important increase in lipolytic activity for pasteurized milk Cheddar cheese during the ripening period occurs between the fifth and twentieth day after making. This increase in lipolytic activity for pasteurized milk cheese occurs considerably earlier in the ripening period than the time at which lipolytic activity is detectable in raw milk cheese. In all pairs of raw and pasteurized milk cheeses examined, the lipolytic activity in the pasteurized milk cheeses was higher on the tenth and twentieth day than in the corresponding raw milk cheeses. It is probable that the factor responsible is related to the differences in flora between raw and pasteurized milk cheese. This early lipolytic activity in pasteurized milk cheese disappears rapidly, as shown in figure 1, and the total lipase content of pasteurized milk cheese at any age after the first 30 days is less than half that of the corresponding raw milk cheese. The bacteria responsible probably are species which are greatly reduced in number during pasteurization.

Since raw milk cheese develops a higher flavor (generally attributed in large part to increased production of volatile fatty acids) than pasteurized milk cheese, it follows that lipases such as those studied in this report are at least in part responsible for more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

SUMMARY

1. The stability and pH activity characteristics of milk lipase are such that this enzyme can play no continuing role in the ripening of Cheddar cheese after making.

2. Analyses made at intervals during the making and ripening of raw milk Cheddar cheese show that milk lipase disappears during the making and is completely absent in the young cheese.

3. The addition of rennet extract during Cheddar cheesemaking causes an increase in lipolytic activity. This increase in lipolytic activity disappears within a period of approximately 30 minutes.

4. After 5 to 20 days, lipases which are considered bacterial begin to make their appearance in the young Cheddar cheese.

5. Most of the lipase active at pH 5 is set free between the fifth and hundredth day of ripening. Lipolytic activity in pasteurized milk Cheddar cheese can be detected in larger amounts between the fifth and twenty-fifth day of the ripening period than in corresponding raw milk Cheddar cheese. At any time after the cheese is 30 days of age, however, the total lipolytic activity of pasteurized milk cheese is less than half that of corresponding raw milk cheese.

6. Bacterial lipases are believed to be at least in part responsible for more rapid flavor development in raw milk Cheddar cheese as compared to Cheddar cheese made from identical milk after pasteurization.

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DETERMINATION OF CHEESE PROTEINASE¹

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During the past several years one of the major problems to be dealt with in the dairy industry has been concerned with the differences in ripening of raw milk and pasteurized milk Cheddar cheese. In an effort to attack this problem from the enzymatic viewpoint, it was necessary, first, to demonstrate the presence of hydrolytic enzymes (both lipases and proteinases) in the cheese, and, secondly, to devise methods suitable for the quantitative determination of these enzymes. This paper deals only with the proteolytic system of cheese. Lipases in cheese have been dealt with in other publications (2, 3).

The presence of proteolytic enzymes in Cheddar cheese has been generally assumed, and adequate evidence of their presence therein is contained in this report.

DETERMINATION OF CHEESE PROTEINASE

Substrate used and its preparation. Casein was chosen as the substrate to be used in the method as finally adopted, not only because it is reproducible but also because it is very similar to the cheese curd itself.

The substrate is prepared as follows: Ten grams of purified casein (Labco Brand, Casein Company of America) are weighed out with an accuracy of ± 0.1 g. To the weighed sample in a Waring Blendor or other suitable mixer, 25 ml. of distilled water and 25 ml. of 1 N sodium hydroxide are added. The power mixer is run at low speed for 2 minutes and then at high speed for 5 minutes. At the end of this time, while the mixer is still running, 40 ml. of a 0.2 molar sodium citrate solution are added and the mixer is allowed to run at high speed for 2 minutes longer. The substrate solution then is diluted to an approximate volume of 300 ml. At this point, while the mixer is running at high speed, 2 g. of gum ghatti dissolved in 30–40 ml. of distilled water are added. The total volume of the mixture should be less than 350 ml. The mixer is allowed to run for 5 minutes after the addition of the gum ghatti. A few drops of methyl red now are added. While the mixer is running, concentrated sulfuric acid is added dropwise until a pH of 5 is reached (red to methyl red). After a pH of 5 is reached, the mixer is allowed to run at high speed for 5 more minutes. At the end of this period the substrate preparation is quantitatively re-

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moved from the mixer and diluted to 400 ml. The suspension obtained is run through a small hand homogenizer three to four times. After homogenization, the suspension is filtered through coarse qualitative filter paper to remove extraneous material and then is ready for use. Since substrate adequate for several enzyme analyses is prepared in the preceding operation, enough toluol to saturate the mixture is added as a preservative. Stock substrate solutions should be rehomogenized and filtered each day before use.

Preparation and analysis of cheese samples for proteinase content.

Twenty grams of the cheese are weighed out with an accuracy of ± 0.1 g. To the weighed sample in a Waring Blender or other suitable mixer, enough distilled water is added to make an approximate volume of 100 ml. The power mixer then is run at low speed for 2 minutes. This length of time is ample to macerate the cheese sample. The mixer then is set at high speed and allowed to run 7 minutes more. At the end of this time the mixture is in the form of a smooth, white cream. This mixture is quantitatively transferred to a 250-ml. graduate with less than 100 ml. of distilled water. The total volume of the mixture then is made to exactly 200 ml. in the graduate. After thorough mixing in the graduate, the suspension is homogenized five to six times through a small hand homogenizer. The cheese sample now is ready to be subjected to enzyme analysis.

In this method for the estimation of proteinase content of Cheddar cheese, a standard suspension of casein is digested under standard conditions, and the undigested casein is precipitated with trichloroacetic acid. The amount of unprecipitated protein split products, which is a measure of the amount of proteinase present, is estimated by measurement of the nitrogen contained therein. The nitrogen content of these unprecipitated protein split products, to be referred to hereafter as non-protein nitrogen, is determined by a colorimetric micromethod (1).

The enzyme analysis as finally adopted proceeds as follows: To 5 ml. of substrate suspension, 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of freshly prepared 0.015 molar cysteine hydrochloride, and 0.2 ml. toluol are added, and the whole is shaken vigorously for 2 minutes. This suspension and the homogenized cheese sample are placed separately in a water bath held at 40° C. When both have attained this temperature, from 0.5 to 3 ml. (depending upon the activity) of the cheese suspension are added to the substrate suspension, and the total volume is made to 10.2 ml. with distilled water. This digestion mixture is shaken vigorously for 30 seconds and then run quickly two to three times through a small hand homogenizer into a small test tube. After the removal of a 1-ml. aliquot for the determination of initial non-protein nitrogen present, the digestion mixture is incubated in a stoppered tube in the water bath at 40° C. for 5 hours. At the end of this time the digestion mixture is rehomogenized as before, and another 1-ml. aliquot is removed for analysis. The difference in non-protein nitrogen

content of the two aliquots represents casein hydrolysis and is a measure of proteinase present.

The analysis of the 1-ml. aliquots for non-protein nitrogen content is carried out as follows: The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 25 ml. of 0.3 N trichloroacetic acid in a large test tube. The tube is shaken vigorously and the contents filtered through a dry filter paper. An 11-cm. filter paper, such as Whatman no. 2, which does not adsorb protein split products, must be used. A suitable aliquot of the filtrate obtained is analyzed for nitrogen content by the colorimetric micromethod (1). The following alternative procedure for nitrogen analysis of the 1-ml. digestion mixture aliquots is used when the cheese samples are low in proteinase content. The 1-ml. aliquot is pipetted immediately upon removal from the digestion mixture into 4 ml. of 1 N trichloroacetic acid in a small test tube. The tube is shaken vigorously, and the contents are filtered through a dry 3-cm. filter paper of the same type as described above. The filtrate obtained is analyzed as above for nitrogen content. It usually is best to run a zero time analysis for each tube in both the above procedures since the non-protein nitrogen blank varies from time to time.

One cheese proteinase unit is defined as the amount of enzyme which, when diluted to 10.2 ml. and incubated for 5 hours under the above conditions, will liberate 1 $\mu\text{g.}$ of non-protein nitrogen. For instance, if 2 ml. of enzyme sample are used per 10.2 ml. of digestion mixture, and 1 ml. of digestion mixture ($1/10.2$ of the total) is added to 25 ml. of trichloroacetic acid (total volume of 26 ml.), and the observed increase in non-protein nitrogen is 7.5 $\mu\text{g.}$ per 5 ml. of trichloroacetic acid filtrate ($5/26$ of the total volume), the number of proteinase units present in the 2-ml. enzyme sample is $7.5 \times 10.2 \times 26 \div 5$ or 397.8 units, or 198.9 proteinase units per ml. of enzyme sample. That the relation between amount of enzyme and increase in soluble nitrogen is linear is demonstrated by the experiments summarized in figure 3 and table 2. In figure 3 the relationship between quantity of cheese proteinase and amount of casein hydrolysis is presented. An arbitrary enzyme preparation was used in this experiment. As may be seen, care should be taken that the amount of enzyme preparation used is such that non-protein nitrogen increases of 2.75 $\mu\text{g.}$ or less per ml. of trichloroacetic acid filtrates are obtained. Above this level the casein hydrolysis obtained is not directly proportional to the quantity of proteinase present.

FACTORS AFFECTING THE ACTIVITY OF CHEESE PROTEINASE

Effect of pH on cheese proteinase activity. In figure 1 the pH activity curve for the hydrolysis of cheese proteinase is given. Determinations of enzyme activity at various pH values were carried out according to the procedure outlined previously in this paper. By means of a composite 0.5 molar

acetate, 0.5 molar phosphate, 0.5 molar borate buffer, pH was controlled throughout the pH range desired. Aliquots of this composite buffer were adjusted with 5 N sulfuric acid or 5 N sodium hydroxide to the pH values shown in figure 1. Proteinase determinations at these pH values were then carried out using in place of 1 ml. of 1 molar acetate buffer at pH 5, 1 ml. of the pH adjusted aliquots of the composite buffer.

It will be seen from the figure that casein is split most rapidly at pH 5. There also is a secondary optimum at pH 7 to 8. Other experiments have shown that this secondary pH optimum seems to vary with the age of the cheese. The primary pH optimum of the proteolytic activity, however, seems to be quite constant throughout the cheese ripening period. The

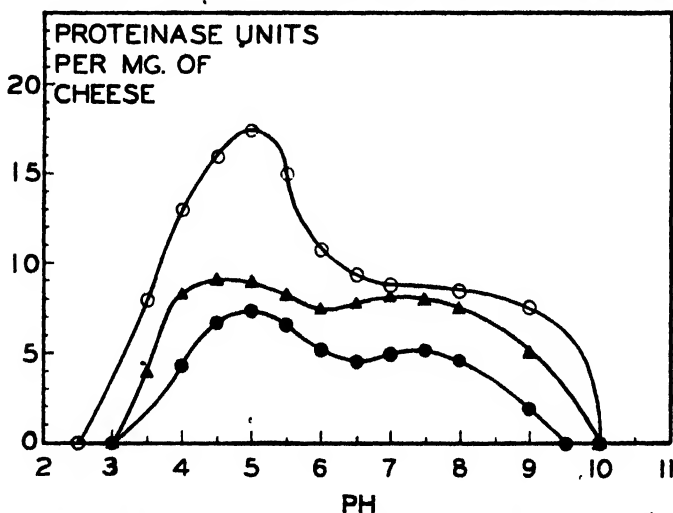


FIG. 1. Effect of pH on rate of casein hydrolysis by cheese extracts. Circles represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 6-month-old raw milk cheese A-100; triangles represent enzyme analyses without cysteine of extracts of 6-month-old raw milk cheese A-100; and dots represent enzyme analyses in the presence of 0.015 molar cysteine of extracts of 2-month-old raw milk cheese A-100.

presence of two peaks in the pH optimum of cheese proteinase indicates strongly that two or more proteinases are present. This also is indicated by cysteine activation of the enzymes at various pH values (fig. 1). The proteolytic activity at pH 5 is greatly activated by cysteine and probably represents endocellular bacterial proteinases liberated by bacterial autolysis (4). The proteolytic activity at pH 7 and above is unaffected by cysteine and probably represents extracellular proteinases liberated by bacteria during their normal life cycle. Since the pH of ripening cheese remains at or very near 5, only the proteolytic activity at pH 5 present in the cheese is important.

TABLE 1

The effects of activators and buffers on cheese proteinase activity

Substance	Concentration	Apparent cheese proteinase in sample
	M	(units/mg. cheese)
Activators		
None		25.9
Potassium cyanide	0.01	64.5
Sodium sulfide	0.01	35.2
Cysteine hydrochloride	0.01	70.8
Buffers*		
Aniline	0.04	29.2†
Pyridine	0.1	21.4
Phosphate	0.1	29.2
Borate	0.1	29.3
Phthalate	0.1	27.3
Acetate	0.1	29.4

* All buffers were adjusted to pH 5 with 5 N sulfuric acid or 5 N sodium hydroxide prior to proteinase determination.

† The proteinase solution used in the buffer experiments was not the same as that used in the activator experiments.

Effect of buffers on cheese proteinase activity. In the development of a method for the estimation of cheese proteinase, it was necessary to find a buffer which did not affect the proteinase activity and which had good buffering capacity in the desired pH range. In table 1 are shown the effects of six buffers upon the activity of cheese proteinase. The acetate buffer appears to meet the requirements and also is convenient.

Effect of activators on cheese proteinase activity. Various reducing agents known to possess definite activating properties for proteinases such as papain were tested for activating effect on cheese proteinase. As may

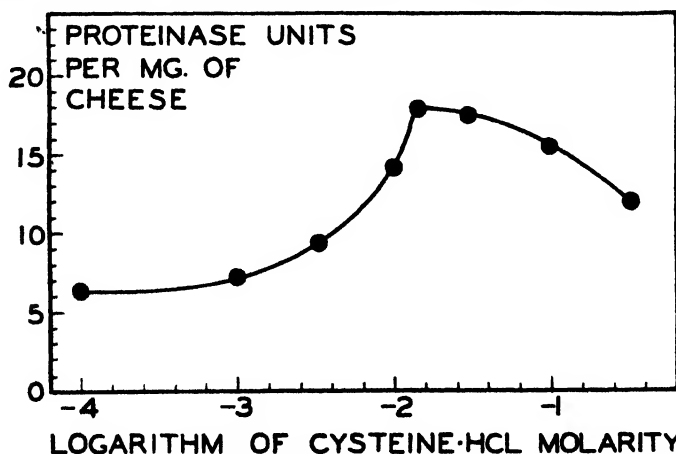


FIG. 2. Effect of cysteine hydrochloride concentration on cheese proteinase activity.

be seen from table 1, activation of the enzyme was obtained in all cases. Cysteine, however, gave more complete activation than sodium sulfide or potassium cyanide.

In order to determine the concentration of cysteine necessary to give maximum activation of cheese proteinase at pH 5, an experiment was run determining the proteinase activity of an enzyme preparation at various levels of cysteine hydrochloride. The results are given in figure 2. It may be seen that maximum activation of cheese proteinase occurred when the

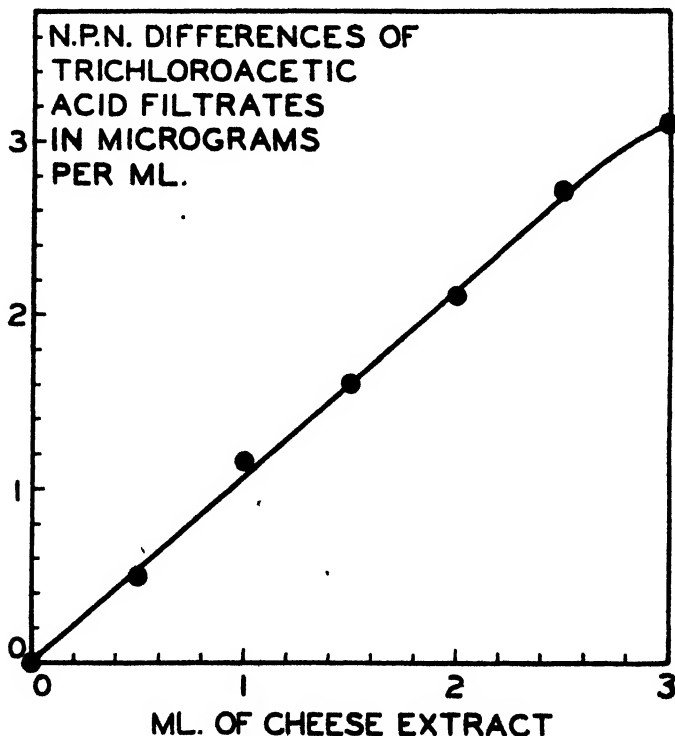


FIG. 3. Relation between quantity of cheese proteinase and amount of casein hydrolysis.

cysteine was present at a level of 0.015 molar. The use of cysteine hydrochloride in order to insure maximum activity of enzyme preparations at pH 5 then was incorporated into the method as outlined under "Preparation and analysis of cheese samples for proteinase content".

Kinetics of cheese proteinase. It already has been shown that the casein hydrolysis obtained is directly proportional to the quantity of cheese proteinase present (Fig. 3). In figure 4 the effect of incubation time on casein hydrolysis by cheese proteinase is presented. It may be seen that the hydrolysis of casein by cheese proteinase at pH 5 is linear up to the time when

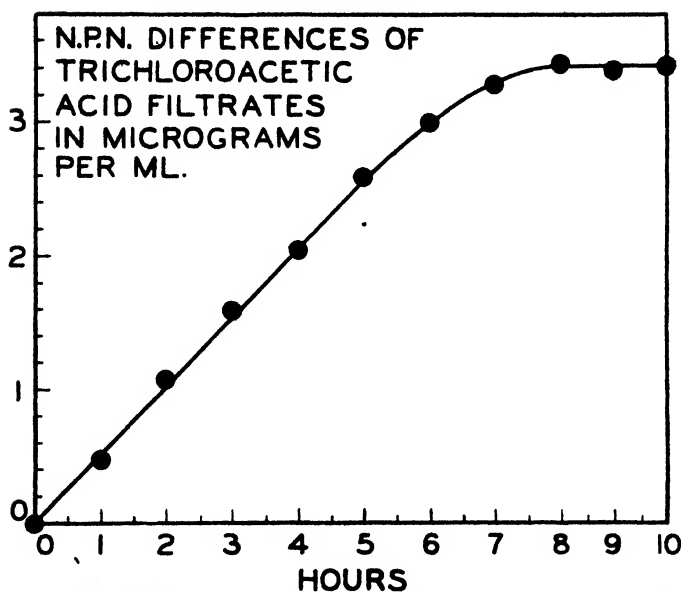


FIG. 4. Effect of incubation time on casein hydrolysis by cheese proteinase.

the increase of protein split products in the trichloroacetic acid filtrate is equivalent to 2.60 μg . non-protein nitrogen per ml.

Reliability of the method. The reliability of the method was checked by running varying concentrations of the same enzyme preparation. The results may be found in table 2. As may be seen, determinations run at various sample levels agree within 10 per cent.

SUMMARY

1. Data are presented showing the presence of proteolytic activity in Cheddar cheese.

2. A method is presented for the quantitative determination of proteo-

TABLE 2

Effect of sample size on apparent cheese proteinase content of a cheese extract

Sample size	Non-protein nitrogen increases in trichloro- acetic acid filtrates	Proteinase content
(ml.)	($\mu\text{g./ml.}$)	(units/ml.)
1.0	0.72	191
1.0	0.79	210
2.0	1.57	208
2.0	1.52	202
3.0	2.36	209
3.0	2.30	203

lytic activity in cheese. Determinations run at various sample levels agree within 10 per cent.

3. Hydrolysis of casein by the Cheddar cheese proteinase system is most rapid at pH 5. A secondary optimum occurs at pH 7 to 8.

4. Proteolytic activity of cheese suspensions at pH 5 is enhanced in the presence of reducing agents.

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PROTEINASE CONTENT OF CHEDDAR CHEESE DURING MAKING AND RIPENING¹

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It is a well recognized fact that proteinases play an important part in the ripening of Cheddar cheese because of their action on the cheese curd. Possible sources of cheese proteinases are the proteinases of milk, of rennet, and of microorganisms. It was first suggested by Babcock and Russell (1) and Babcock *et al.* (5) that an inherent milk proteinase, termed galactase, probably is the most important proteolytic agent in cheese ripening. Later work on variation of rennet levels in cheesemaking caused Babcock *et al.* (3) to revise their early views. Their final conclusion was that rennet is the most important proteinase source in cheese, its protein digestive action being due to the action of pepsin present in the rennet as an impurity. Sherwood (12), however, reported that the use of pepsin for rennet in Cheddar cheese resulted in 40-50 per cent less protein degradation than took place in normal rennet control cheeses. The ratios between amounts of different protein split products were quite similar for both types of cheese, indicating that nitrogen partition was identical. Sherwood (11) also found that although the extent of protein degradation in chloroform-treated Cheddar cheese was considerably less than for normal Cheddar cheese controls, the general course of nitrogen partition was the same for both chloroform-treated and normal cheese. The same work showed that normal Cheddar cheese had consistently higher levels of subpeptone nitrogen than chloroform-treated cheese. From these data Sherwood concluded that rennet itself is the most important proteolytic agent in Cheddar cheese ripening, and since its action extends but little beyond the peptone stage, any further proteolytic degradation must be due to bacterial action.

The purpose of the present investigation is to establish the relative importance of various sources of cheese proteinases, and also to present studies of the differences in proteolytic activity during the making and ripening of raw and pasteurized Cheddar cheese made from the same milk.

METHODS

Cheese proteinase. The method used to determine proteolytic activity in Cheddar cheese reported by the present authors (10) was used in all cheese proteinase analyses. All analyses were made both with and without 0.015

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molar cysteine in order to differentiate between cysteine-activated proteinases and those unaffected by cysteine.

Cheesemaking procedure. The milk used in making the experimental cheeses was mixed, raw, whole milk from the University Dairy. A 920-lb. lot of the milk was divided into two equal portions. The first portion received no treatment. The second portion was subjected to pasteurization (holder method—30 min. at 145° F.). Each portion, after treatment, was placed in a separate vat, and both received identical subsequent treatment. Cheeses were made by an experienced maker according to standard procedures. From each vat two 22-lb. cheeses were obtained. The green cheeses produced were kept at 68° F. for a period of 2–3 days and then were transferred to a cold room (40–45° F.) for the remainder of the ripening period.

Sampling. Samples for enzyme analysis were taken at various intervals during the making process and ripening period. Sampling times used are indicated in the figures and tables.

PROTEOLYTIC ACTIVITY OF CHEDDAR CHEESE DURING MAKING AND RIPENING

Proteinase content of raw and pasteurized Cheddar cheese. In figures 1-a, 1-b, 2-a, and 2-b the averages of the proteolytic activities at pH 5 of a series of pairs of pasteurized milk cheeses and raw milk cheeses made by the procedure described under "Methods" are given. Figures 1-b and 2-b cover the entire ripening period. Figures 1-a and 2-a show, on an enlarged scale, the changes occurring during the making and the first few days of ripening.

Figures 1-a and 1-b, representing the proteolytic activity in raw milk Cheddar cheese, will be considered first. In figure 1-a, it will be noted that a small amount of proteolytic activity is present in the milk at the time it is placed in the vat. This represents milk proteinase, the significance of which will be discussed later. The first important increase in proteolytic activity occurs 90 to 120 minutes after the start of making. While part of this increase can be attributed to proteinases of the added rennet, comparison of figures 1-a and 2-a indicates that most of this increase probably is due to bacteria growing and producing extracellular proteinases during the making process. Since the corresponding increase in the proteolytic activity of pasteurized milk cheese (fig. 2-a) is much smaller, it is believed that the bacteria responsible are destroyed in the pasteurization process. No further change in proteolytic activity occurs until after the cheese is in its final form and has been stored. In the first few days of ripening a large increase in cysteine-activated proteinases occurs. The use of cysteine here is valuable in that it affords a means of differentiation in the type of proteinases present in the cheese and thus affords a clue to the source of these

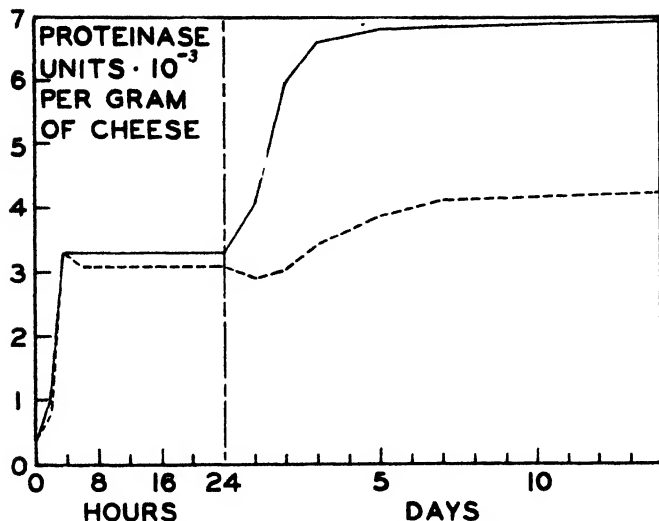


FIG. 1-a. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

proteinases. It should be emphasized that in the ripening cheese where strictly anaerobic conditions are present (6), the cysteine-activated proteinases undoubtedly are present in a fully active state.

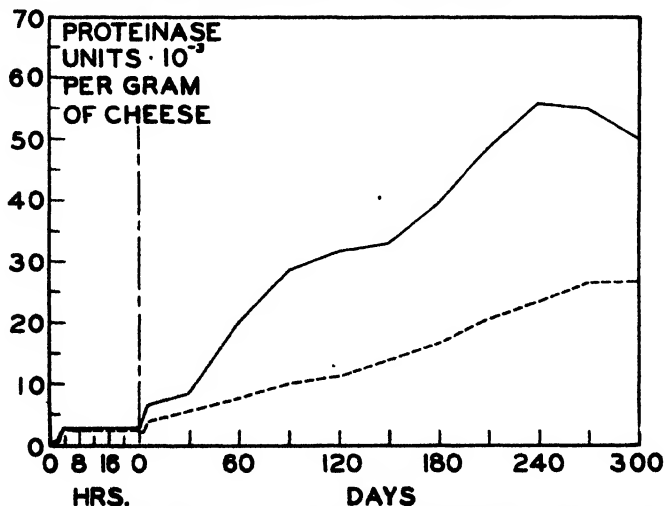


FIG. 1-b. Proteinase activity at pH 5 in raw milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 1-a. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

Figures 1-a and 1-b show that the amount of proteinase in the milk is very small compared to the proteinase of the cheese. Consequently, even if the milk proteinase is carried over quantitatively into the cheese, its role in ripening must be small. It also will be noted that the proteolytic activity at pH 5 of the added rennet is relatively small; it is readily seen, therefore, why such large amounts of rennet extract must be added to Cheddar cheese in order to increase markedly the rate of ripening (2, 3, 4, 7, 8, 9, 14).

Figure 1-a indicates that most of the proteinase of the young cheese is bacterial in origin. Part of this is produced in the vat, and the remainder is set free during the first few days of ripening. Figure 1-b shows that as

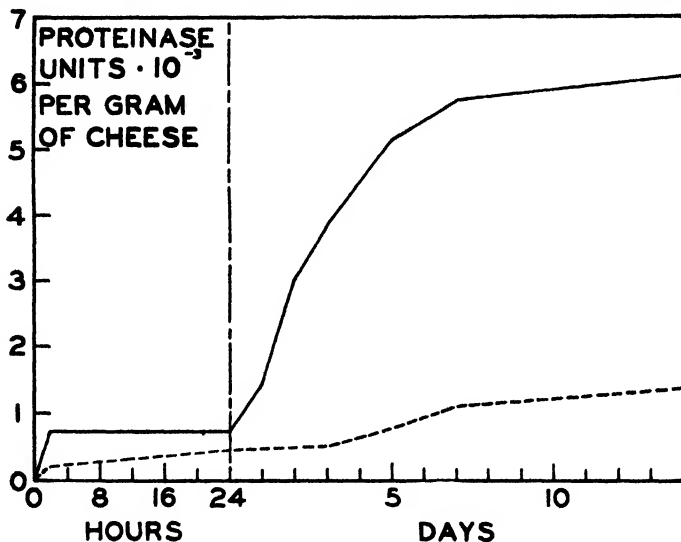


FIG. 2-a. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and the early stages of ripening. The curves represent the average of six cheeses. Solid line, with cysteine; broken line, without cysteine.

the cheese ripens its proteinase content increases greatly. It is very apparent that only a small portion of the total proteolytic activity is contributed by the milk and the rennet when standard Cheddar cheesemaking procedures are employed. The greater portion of the proteinase increase is cysteine-activated and may represent endocellular bacterial proteinases liberated by bacterial autolysis (13).

The proteinase activity of pasteurized milk cheese at various ages is shown in figures 2-a and 2-b. In pasteurized milk cheese (fig. 2-a) there is no increase in proteinase in the vat other than that introduced with the rennet. There is a relatively large increase in cysteine-activated proteinase during the first few days of ripening. Figure 2-b shows that the total proteinase content of pasteurized milk cheese during ripening is less than that

of raw milk cheese, and that in pasteurized milk cheese much less cysteine-activated proteinase is present.

Since the sources of the proteinases found in Cheddar cheese during the later ripening period must be in large part bacterial, it might readily be assumed that some of the differences between raw and pasteurized milk cheese made from the same lot of milk could be attributed to this difference in cysteine-activated proteinase content. The bacteria responsible probably are species which are greatly reduced in number during pasteurization. It also may be seen from figures 1-*b* and 2-*b* that the content of proteinase, not activated by cysteine, becomes greater in the pasteurized milk cheese than

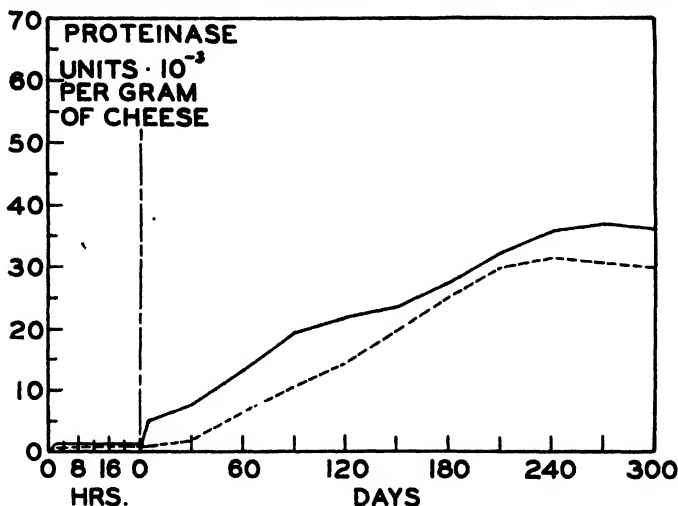


FIG. 2-*b*. Proteinase activity at pH 5 in pasteurized milk Cheddar cheese during making and a 300-day ripening period, same cheeses as Fig. 2-*a*. The analyses after 180 days, however, were made on only one pair of these cheeses. Solid line, with cysteine; broken line, without cysteine.

that of the raw milk cheese after 80 days of ripening and remains greater throughout the rest of the ripening period.

During ripening, flavor development always is more complete in raw milk cheese than in pasteurized milk cheese of the same age. Since the difference in proteinase content between the two types is largely in the cysteine-activated fraction, it follows that cysteine-activated proteinases may be responsible, in part, for the more rapid flavor development in raw milk cheese. Since the organisms responsible are present in raw milk but are largely destroyed in pasteurization, it appears that improvement in pasteurized milk Cheddar cheese might be obtained if these organisms could be isolated, characterized, and added with the starter culture.

Reproducibility of cheeses. The data of figures 1 and 2 represent aver-

TABLE 1
Proteinase content of Cheddar cheese during ripening

Cheese no.*	Proteinase content in units per milligram of cheese									
	With cysteine†					Without cysteine				
	0 days	10 days	30 days	90 days	180 days	0 days	10 days	30 days	90 days	180 days
1015R	3.5	6.6	8.2	27.5	36.6	2.6	4.1	5.1	9.5	16.3
1022R	3.0	6.2	7.5	21.3	33.1	2.1	3.8	4.8	9.2	15.8
1103R	3.9	7.0	8.3	30.1	42.7	2.5	4.3	5.0	9.8	17.0
1119R	4.1	7.0	8.4	31.2	41.7	2.8	4.5	5.3	10.0	17.4
1124R	3.6	6.7	8.1	29.0	41.3	2.6	3.9	5.1	9.6	16.5
1201R	3.5	6.4	8.0	28.0	34.8	2.4	3.7	5.0	9.6	15.2
1015P	0	5.5	7.2	18.0	25.8	0	1.1	1.9	9.3	27.2
1022P	0	4.9	7.7	17.5	22.1	0	0.8	1.4	10.0	26.5
1103P	0	5.7	7.6	18.9	29.1	0	1.2	1.5	10.1	28.1
1119P	0	6.0	7.9	17.4	28.5	0	1.5	2.1	10.4	30.1
1124P	0	5.6	7.0	17.7	24.9	0	1.0	1.6	8.9	26.2
1201P	0	5.4	7.2	19.9	22.9	0	1.0	1.0	8.7	24.7

* The letter R indicates a raw milk cheese, P a pasteurized milk cheese. Cheeses made from the same lot of milk have the same number.

† Proteinase determinations were made at pH 5 both with and without 0.015 M cysteine (10).

ages of six pairs of raw and pasteurized milk cheeses. Analyses after 180 days, however, were made on only one pair of cheeses. The individual curves for the six pairs of cheeses were very similar. The individual analyses are presented in table 1 and may be seen to check closely.

SUMMARY

1. The proteinase content of a series of pairs of raw and pasteurized milk Cheddar cheeses has been determined at intervals during the making and ripening periods.

2. The active proteinase in ripening Cheddar cheese is largely of bacterial origin; only a small fraction of the total activity is contributed by the milk and the rennet.

3. Pasteurized milk Cheddar cheese is characterized by a lower content of cysteine-activated proteinase than raw milk Cheddar cheese.

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THE HERITABILITY OF OFFICIAL TYPE RATINGS AND THE CORRELATION BETWEEN TYPE RATINGS AND BUTTER-FAT PRODUCTION OF AYRSHIRE COWS¹

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The selection of dairy cattle for breeding purposes usually is based on the type and the production records of the animals, their ancestors or their progeny. The consideration given to type in a selection program should be determined by its economic importance to the breeder—i.e., by the advertising value and the increase in selling prices of animals with high classification ratings, by the relationship between type and production, and by the heritability of type. The economic importance of type varies from herd to herd, depending on the sales policy used by the breeder. Neither the correlation between type and production nor the heritability of type should vary much from herd to herd within a breed.

The purpose of this study was to estimate the heritability of single type ratings and to compute the correlation between type and production in Ayrshire cattle.

EXPERIMENTAL PROCEDURE

The data used in this study were the type ratings of Ayrshire cows classified between March, 1942, and May, 1946, by official inspectors in accordance with the rules of the classification program of the Ayrshire Breeders' Association. The participation of Ayrshire herds in this program is voluntary. According to the plan, the breeder submits for official inspection all of his cows that have freshened one or more times. These cows are given a single rating of one of five grades: Excellent, Very Good, Good Plus, Good, and Fair.

The rating and other information on each cow were coded and punched on International Business Machine cards. These cards were sorted according to inspector and date of classification. The correlation coefficients between ratings of related animals that were classified on the same day by the same inspector were computed and used to estimate the proportion of the total variability in ratings that is transmitted from parents to their offspring. In this paper this transmitted portion is called the heritability. From the correlation between the type ratings of paternal sisters and the regression of daughter's rating on dam's rating after removing the influence of the sires, the heritability of single type ratings was estimated.

The butterfat production records of classified cows were used to calculate

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the correlation between type and production. Type was paired with the first record, with the record begun nearest to the date of classification, and with the average of all records. All butterfat records were adjusted to a mature-equivalent, 305-day, twice-a-day milking basis.

RESULTS

Heritability of type. The classification ratings of 3,738 cows sired by 368 bulls were used to compute the correlation between the ratings of paternal sisters. Only those sires with six or more daughters that were classified the same day were included in this study. This procedure should have eliminated most of those sires which would be represented by a few highly selected mature daughters. The frequency distribution of the ratings of these 3,738 cows and the distribution of 8,573 Ayrshire cows that were classified during the same period are given in table 1. The agreement between the two columns showing percentages indicates that the group of 3,738 cows is a representative sample of the breed.

TABLE 1

Frequency distribution of the ratings for paternal sisters and for the Ayrshire breed

Rating	No. of paternal sisters	Percentage of total	Breed percentage of total*
Excellent	109	2.9	3.7
Very good ..	831	22.2	22.1
Good plus .	1627	43.6	42.7
Good	987	26.4	26.8
Fair	184	4.9	4.7
Total	3738	100.0	100.0

* This is the distribution of the classification ratings of 8,573 Ayrshire females classified between March, 1942, and May, 1946.

The variance of these type ratings was divided into variation between inspectors, between sires within inspectors, and between daughters by the same sire, as shown in table 2 (9). The standard deviation of all ratings was 0.9. The variance component *A* represents variation between daughters by the same sire, while component *B* is the additional variance which can be ascribed to difference between sires, and *C* to differences between inspectors. The ratio $\frac{B}{A+B}$ is the average correlation between daughters by the same sire. In these data this correlation was 0.12 with a standard error of 0.04.

There were 1,601 cows whose dams were classified on the same day by the same inspector. These cows were sired by 789 sires. The analysis of covariance technique was used (table 3) to obtain the regression of daughter's rating on dam's rating within sires and this coefficient was 0.14 ± 0.034 . The regression of offspring on dam within sires avoids much

TABLE 2
Analysis of the variation in type ratings of 3,738 cows sired by 368 bulls

Source of variation	Degrees of freedom	Mean square	Variance components of mean square*	Individual components
Between inspectors	9	15.330†	$A + 10.16 B + 373.7 C$	$C = 0.037$
Between sires within insps.	358	1.634†	$A + 10.16 B$	$B = 0.095$
Within sires within insps.	3370	0.667	A	$A = 0.667$
Summary	3737	0.795	$A + 0.998 B + 0.9 C$	$A + B + C = 0.799$
			$\frac{B}{A+B} = 0.12$	

* Coefficients for the components of variance were computed by the method used by Dickerson (4).
 † $P < 0.01$.

of the environmental contribution which would be found in a gross correlation or regression between parent and offspring.

Estimates of the heritability² of type in cattle can be made from the above relationship. In a random breeding population the correlation between traits of paternal sisters may be expected to contain 0.25 of the additive genetic variance, 0.06 or less of the epistatic variance, plus the environmental portion of the variance times the correlation between the environments of the half sisters (10). However, in those herds where the only source of new stock is through sires, the average genetic relationship between two animals by the same bull often is greater than 0.25. In most cases the animals used in this study were from herds using only one or two sires. Therefore, the average genetic relationship of the paternal sisters should be approximately 0.30. Since the environmental contribution to the

TABLE 3

Analysis of covariance of type ratings of dams and daughters and the intra-sire regression of daughter's rating on dam's rating

Source of variation	Degrees of freedom	Sums of squares		Cross products	Regression dau. on dam
		Daughters	Dams		
Between sires	788	719	830	240	0.14
Within sires	812	452	481	66	
Total	1600	1171	1311	306	

paternal sister correlation could not be determined accurately, the heritability of type ratings could be estimated to be somewhat less than $\frac{1}{0.30} \times 0.12$ or 0.40 from these data.

The intra-sire regression coefficient of daughter's rating on dam's rating should contain 0.5 of the additive genetic variance plus about 0.25 of the epistatic variance. Non-random matings would not affect the intra-sire regression coefficient of daughter on dam (6). Hence, the estimate of heritability from these data would be about 2×0.14 or 0.28.

When the two estimates of heritability are combined, the average figure is 0.30 and the 95 per cent fiducial limits are 0.19 and 0.42. This means that the chances are very good (19 out of 20) that the real heritability of type ratings lies within these limits.

Correlation between type and production. The official ratings, number of cows, percentage of total, and average butterfat production (first record, record started nearest to the date of classification, and the average of all records) of the 5,177 cows that were classified and also had production

² Lush defined the degree of heritability as "the fraction of the observed variance which was caused by differences in heredity" (6). This fraction may result from additive gene effects, interaction of allelic genes (dominance), and interaction of non-allelic genes (epistasis).

records are given in table 4. On the basis of the percentages for the classification groups for all classified Ayrshires (table 1), a larger percentage of the Excellent and Very Good cows have production records. This is similar to Copeland's findings (1, 2) in his study of classified Jersey cows, and means either that the herds on official test were culled more closely before the inspector classified the cattle, that these herds were inherently better in type conformation than the herds that were not on test, or that inspectors are influenced by the records (consciously or unconsciously) and thereby place higher producers in the Excellent and Very Good classes when they know about production and do not when the production is unknown to them.

The butterfat production of cows increased with each rise in type rating with the exception of the average of the first butterfat records of the Good and Fair groups of cows.

In order to remove the effect of herd on the correlation between type and

TABLE 4

Frequency distribution of the ratings of the cows with production records and the average production of butterfat for each rating

Classification rating	No. of cows	Percentage of total	Av. butterfat production		
			1st records	Nearest record	Av. all records
Excellent	320	6.2	415	417	412
Very good	1479	28.6	394	385	386
Good plus	2105	40.6	376	367	369
Good	1097	21.2	361	352	353
Fair	176	3.4	363	345	350
Summary	5177	100.0	380	371	372

butterfat production, the data were analyzed by the analysis of covariance to obtain the intra-herd correlation and regression of butterfat production on type rating. The correlation and regression coefficients of the first, nearest, and average of all butterfat records on type rating are given in table 5. The between-herd correlation coefficients, $r = 0.53, 0.42$, and 0.50 , are highly significant and mean that those herds classified as the best type tended to have the highest average production per cow. Within each herd, however, the correlation coefficients between type and butterfat production were lower, $r = 0.16, 0.16$, and 0.19 , but still statistically significant. The regression coefficients show that within a herd the butterfat production of the cows increased about 12-14 lb. for each increase in type grade. However, herds that averaged one classification grade higher in type also averaged about 82 lb. more butterfat per cow.

DISCUSSION

The objectives of this study in dairy cattle breeding were to determine the heritability (transmitted portion of the superiority of parents over

average of group from which parents were selected) of type and the correlation between type and production. On the basis of the studies of economic characteristics in swine (3), and milk and butterfat production in dairy cattle (8), it would seem that the results found here might well apply to other breeds of dairy cattle.

The results have shown that the heritability of single type ratings in Ayrshire cows probably lies between 0.19 and 0.42. This indicates that selection for type through a type classification program should improve the type of future generations of dairy cattle. If type was considered in the selection program, the breeder could expect the next group of offspring from the selected parents to be approximately the heritability times the difference between the type of the selected parents and the type of the group or herd from which the parents came. For example, if the average type of a herd was Good Plus (82.5) and the animals selected as parents averaged Very

TABLE 5

Analysis of covariance and the correlation and regression coefficients of butterfat production on type rating

Source of variation	Degrees of freedom	Correlation coefficients*			Regression coefficients*		
		1st record & type	Nearest record & type	Av. all records & type	1st record on type	Nearest record on type	Av. all records on type
Between herds	303	0.527	0.417	0.497	82.6	80.3	83.2
Within herds	4873	0.156	0.159	0.193	11.6	13.9	12.9
Summary	5176	0.193	0.184	0.220	15.7	17.7	16.8

* $P < 0.01$ for all values found.

Good (87.5), then the best estimate of the type of the offspring of these parents is $82.5 + 0.2(87.5 - 82.5)$, or 83.5 if the heritability of type is 0.2, and 84 and 84.5 if the heritability is either 0.3 or 0.4, respectively. Actually, the improvement in type would not be this fast unless the sire's breeding value for type was as equally well known as the type of the cows selected as parents. Estimates of a sire's breeding value for type could be made from a progeny test or from a pedigree study. The correlation between the type of a sire and the type of his daughters is not well known, although Copeland's figures indicated a low relationship (1).

The intra-herd relationship of type and butterfat production was 0.16 between type and single records, and 0.19 between type and average of all records. These correlation coefficients are very similar to those reported by Lush (7) for Holstein-Friesian cows, but somewhat smaller than the coefficients which Copeland (2) computed between type and butterfat records of Jersey cows. The difference between the correlation coefficient between type and a single butterfat record (first or nearest) and the correlation

coefficient between type and the average of all butterfat records is not statistically significant, but it is in the expected direction.³ Intra-herd correlations were computed for this study because in most cases selection is practiced among animals that are raised on the same farm. Selection within herds for type only would not result in any substantial increase in butterfat production. Likewise, selection for butterfat alone would not increase the type of the animals to any great extent.

Hazel (5) has described a procedure to derive selection indexes. Before an index can be made for butterfat and type, however, the relative economic importance of each must be determined, and likewise the correlation between the genotypes (breeding value) for type and butterfat must be known. The advantage of an index is that improvement would be made in both type and production so that the gain would mean most to the breeder in dollars and cents. It seems probable that other characteristics (such as longevity and reproductive behavior) should be considered in deriving an index for selecting dairy cattle.

SUMMARY

The classification ratings of 3,738 paternal sisters and of 1,601 cows out of classified dams were used to estimate the portion of the differences in official type ratings that is transmitted from parents to offspring. This portion is called heritability and from these data this figure is estimated to be 0.3. Thus offspring inherit about one-third of the observed superiority of the parents' type, and herd improvement in the type of dairy cattle can be made by the selection of animals that are above the herd average in type for parents of the next generation.

The correlation between classification rating and production of butterfat was computed from data on 5,177 cows. Within herds there was an average increase of 13 lb. of butterfat for each increase of one grade of type. The relationships between the classification rating and the first butterfat record of a cow, between the type rating of a cow and her butterfat record begun nearest the date of classification, and between the type rating of a cow and the average of all her butterfat records were statistically highly significant, but they were so low that their practical significance is quite small. The correlation coefficients were 0.16, 0.16, and 0.19, respectively.

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³ The average number of records per cow was 3.5. If the repeatability of butterfat records is assumed to be 0.4, then the expected correlation between one type rating and the average of all butterfat records is 0.16×1.32 or 0.21.

Economics Department, West Virginia University, and Miss Margaret Cross for the sorting and tabulation of the International Business Machine cards. They also are indebted to Dr. H. O. Henderson, Head, Department of Dairy Husbandry, West Virginia University, and Dr. A. B. Chapman, Department of Genetics, University of Wisconsin, for their valuable suggestions in the preparation of this manuscript.

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VARIATIONS IN TYPE RATINGS OF INDIVIDUAL AYRSHIRE COWS¹

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Type classification programs have been adopted by all the major dairy cattle breed associations. With the exception of the Guernsey breed, in order to participate in this program each breeder must submit all of the registered cows in his herd for classification. At a subsequent reclassification of the herd new ratings on previously classified cows will be official only if the new rating is higher than the previous official rating. Before a definite appraisal of the type classification program can be made, it is desirable to know as much as possible about the amount of variation that occurs when the same cows are classified several times throughout a lifetime. This investigation sought to determine: (a) The degree or amount of variation that occurs when the same cows are classified several times in their lifetime, (b) the degree of agreement between ratings given the same cow by the same inspector and by different inspectors at different times, (c) the possibility that age of animal, stage of lactation, and condition of animal influence the official type rating, (d) the value of photographic records in studying the type of animals from birth to maturity, and (e) the reasons why the ratings of some cows undergo large changes.

EXPERIMENTAL PROCEDURE

The data for this investigation were collected from the Ayrshire herd (known as the Reymann Memorial Herd) at the West Virginia Agricultural Experiment Station. This herd represents approximately 25 years of a continuous, carefully controlled breeding project. No females have been brought into the herd in that period of time, and each normal female that is dropped in the herd must be raised and retained through at least one 305-day lactation before her disposal. The feeding and management of the herd has been kept as nearly constant as possible over the 25-year period. Each animal is bred to freshen each year, allowing 6 to 8 weeks for the dry period.

From 1942 through 1946 all the females that had calved were rated for type approximately three times yearly. During this period 138 cows were rated. Of this number, 32 were rated once, 26 twice, 17 three times, 8 four times, 6 five times, 9 six times, 12 seven times, 9 eight times, 5 nine times, 4 ten times, 2 eleven times, 3 twelve times, and 5 thirteen times. In

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TABLE 1
Classification ratings of cows in the Reymann Memorial Herd rated three times or more from May 16, 1942, to December 20, 1946

Cow no.	Inspector and date													Range
	E 5-42	E 10-42	S 1-43	R 5-43	T 7-43	U 10-43	U 1-44	V 4-44	W 12-44	X 4-45	Y 12-45	T 5-46	Z 12-46	
462	3*	3	3	4	3	3	3	5						1
473	5	5	5	5	5	5	5	5						0
491	4	4	3	4	4	4	4	3						1
500	4	5	5	5	5	5	5	5						1
501	4	4	4	5	5	5	5	5				5	4	2
521	4	4	4	5	5	5	5	5						3
526	3	3	3	3	3	1	4	4						2
530	4	4	4	4	4	4	4	4						1
537	5	4	4	5	4	4	4	4						1
539	4	4	4	4	4	4	4	4						1
581	4	4	4	4	4	4	4	4						1
589	2	4	4	4	2	4	4	4						1
577	4	4	4	4	4	4	4	4						2
592	3	4	4	4	4	4	4	4						1
611	3	3	3	4	4	4	4	4						1
612	3	3	3	4	4	4	4	4						1
614	3	3	3	4	4	4	4	4						1
616	3	3	3	4	4	4	4	4						1
617	4	3	4	4	3	4	4	4				4	4	1
620	3	4	4	4	4	4	4	4						1
622	2	2	3	4	3	3	3	3				2	3	2
624	2	2	3	2	3	3	3	4						2
627	2	2	3	2	3	3	3	4						1
638	2	3	3	2	4	3	3	4						2
640	3	3	3	3	3	3	3	4						2
641	3	3	3	3	3	3	3	4						1
642	3	3	3	3	3	3	3	4						2
644	2	3	3	3	3	3	3	4						0
657	2	3	3	4	4	4	4	5						2
660		3	3	4	4	4	4	5						1
661		2	3	4	4	4	4	5						2
662		2	3	4	4	4	4	5						0
664		2	3	4	4	4	4	5						2
665		2	3	4	4	4	4	5						1
666		3	3	4	4	4	4	5						1
668		3	3	4	4	4	4	5						0
669		3	3	4	4	4	4	5						2
671		3	3	4	4	4	4	5						2
672		3	3	4	4	4	4	5						2
673		3	3	4	4	4	4	5						1

TABLE 1 (Continued)

Cow no.	Inspector and date													
	E 5-42	B 10-42	S 1-43	R 5-43	T 7-43	U 10-43	U 1-44	V 4-44	W 12-44	X 4-45	Y 12-45	T 5-46	Z 12-46	Range
674	..	4	3	3	3	3	3	4						1
675	3	3	2	2	3	2	2						1
676	4	3	3	2	4	3	3						0
677	2	2	2	2	2	3	2						2
678	2	2	2	2	2	3	2						1
679	1	1	1	1	3	3	2						1
680	3	3	3	3	3	2	2						1
681	2	2	2	2	4	4	2						1
683	2	4	4	4	3	3	2						3
684	3	3	3	3	4	2						3
685	3	3	3	2	2	2						1
686						1
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702						1
703						1
704						1
705						1
707						1
714						1
715						1
716						1
719						1
732						1
737						1
743						1
744						1
747						1
754						1
755						1

* Eating code: 5, Excellent; 4, Very Good; 3, Good Plus; 2, Good; 1, Fair.

each instance the type rating was given by an official inspector appointed by the Ayrshire Breeders' Association, who also indicated on a scorecard the main criticisms of each animal classified. The inspectors had no knowledge of any previous ratings on the animals until they had rated each animal and completed their work. The type rating standards of the official type classification program of the Ayrshire Breeders' Association—Excellent, Very Good, Good Plus, Good, and Fair—were used as the standard grades. In order to facilitate the analysis of the data, the official grades were coded, using 5 for Excellent, 4 for Very Good, 3 for Good Plus, 2 for Good, and 1 for Fair.

RESULTS

Variations in individual ratings. Eighty animals were officially rated three times or more. These ratings are shown in table 1, along with the dates classified, the inspector, and the range in the classification ratings for each cow.

A summary for the table shows that seven animals or 8.8 per cent were rated the same each time they were classified, 47 or 58.7 per cent had a range of one grade, 23 or 28.8 per cent had a range of two grades, and 3 or 3.7 per cent varied three grades. These results indicate that approximately one-third of the 80 head varied two or more grades when classified an average of five times over a period of 4.5 years by different inspectors. A further examination of the data was made to determine the major reasons for variations in type ratings.

Correlation between type ratings of the same cow when classified by the same inspector and by different inspectors. The relationships (repeatabilities) between ratings given a cow by the same inspector and between ratings given a cow by different inspectors were computed after the data first were analyzed by the analysis of variance (3). The mean squares for between cows and within cows were obtained and the components of variance for each mean square were determined. This analysis is shown in table 2, where the variation in type ratings of 53 cows classified by inspector R has been analyzed into differences between cows and within cows and the two components of variance, *A* and *B*, are calculated. The component of variance *A* is the variance caused by differences between ratings on the same cow, while *B* represents the additional variation that can be ascribed to differences between cows. The ratio $\frac{B}{A+B}$ represents the average correlation between the ratings on each cow classified by R and is 0.73. Inspector R classified the cows in May, 1942, October, 1942, and May, 1943.

Similarly, the correlations of repeatabilities of ratings of the same cow when classified by the same inspector were 0.82 and 0.62 for inspectors U

TABLE 2

Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by inspector E

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows	52	107.2	2.062	$A + 2.47 B$	$B = 0.727$
Within cows	78	20.8	0.267	A	$A = 0.267$
Total	130	128.0	0.985	$A + 0.988 B$	$A + B = 0.994$
				$\frac{B}{A + B} = 0.731$	

*Coefficients for the components of variance were computed by the method used by Dickerson (1).

and T, respectively, based on 42 and 9 cows. The classifications by inspector U were made in October, 1943, and January, 1944, while inspector T rated the cows in July, 1943, and May, 1946.

In table 3 the analysis of variance was used on the ratings of cows classified two or more times by different inspectors. In this analysis only the first rating placed on an animal by an inspector was used, and any later ratings given to the cow by the same inspector were omitted. The repeatability of ratings for these 101 cows was 0.55.

Tables 1, 2, and 3 indicate that there is a considerable amount of variation in the type ratings of individual Ayrshire cows. Some of the possible reasons for these variations, other than the difference in inspectors, were studied.

Effect of age on variation of type ratings. From observation it appeared that as cows advanced in age there was a tendency for the inspectors to raise their rating. In other words, it seemed that when an animal was classified at an early age, the inspector would rate the animal a grade lower if there was any doubt in his mind, because the animal always could be raised at a subsequent reclassification, but never officially lowered. Table 4 gives the average rating of all the cows classified at the ages of 2 to 11 years, inclusive.

TABLE 3

Analysis of variance of type ratings of cows classified in the Reymann Memorial Herd by different inspectors

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance components of mean square*	Individual components
Between cows	100	255.7	2.557	$A + 4.446 B$	$B = 0.484$
Within cows	348	140.1	0.403	A	$A = 0.403$
Total	448	395.8	0.883	$A + 0.992 B$	$A + B = 0.887$
				$\frac{B}{A + B} = 0.546$	

*Coefficients for the components of variance were computed by the method used by Dickerson (1).

TABLE 4

Average type rating by age for all animals classified between the ages of 2 to 11 years, inclusive

Age classified	No. of animals	Av. rating
2	37	2.27
3	204	2.83
4	127	3.01
5	82	3.35
6	48	3.48
7	33	3.61
8	32	3.69
9	22	3.91
10	23	4.26
11	18	4.06

This table might lead one to believe that as the animals advanced in age the rating went up, but the factor of selection must be considered. The better type animals, as a rule, were kept in the herd longer, and those cows that were classified at 5 years and over were a select group of individuals. Averages as given in this table do not present an accurate picture of the effect of age on the variation of individual type ratings.

A more accurate picture is presented when the effect of age on type rating is studied within the same animals. In this study 65 females were included that were classified numerous times as 3-, 4-, and 5-year-olds. Thirty of the same females were classified several times as 4- and 5-year-olds, and 27 of them classified several times as 3-, 4-, and 5-year-olds. The results of this study are presented in table 5 and indicate that the change in type rating due to advancement in age is not great but is statistically significant between the ages of 4 and 5. More years of results will be needed to make the same determinations for older animals.

Effect of stage of lactation on variation of type ratings. During each calving interval a cow usually undergoes a constant change as far as her physical appearance is concerned. In the majority of cases dairy cows put on flesh during their dry period and when they come into production they are usually well covered with flesh and have a certain bloom that they lose during a heavy-producing lactation. The additional fleshing may serve to

TABLE 5

Average type rating by age within cows

Age classified	No. of animals	Av. rating
3	65	2.94
4	65	2.90
4	30	3.05
5	30	3.24
3	27	2.98
4	27	3.02
5	27	3.21

cover up certain faults; on the other hand, it may cause certain cows to appear to be lacking in dairy temperament. The appearance of the udder also changes constantly.

In an effort to determine some specific measure of the effect of stage of lactation on the variation of type ratings of the same cow, an analysis was made of the ratings of 63 animals classified 137 times between freshening and the fourth month of lactation, inclusive, 140 times between the fifth and the ninth month of lactation, inclusive, and 173 times between the tenth month of lactation and the next freshening period. The average of the ratings made between freshening and the fourth month, inclusive, was 3.27; between the fifth and the ninth month, 3.08; and from the tenth month of lactation to the next freshening period, 3.35. These differences are small, but are statistically significant when either the early or the last part of the lactation is compared with the middle segment. The data suggest that a little higher rating is obtained when animals are classified shortly before or after freshening, rather than in the middle part of their lactation.

As yet data are insufficient to make monthly comparisons of ratings throughout the lactation of a number of animals, which would be most desirable. Individual differences are great when cows are compared as to their changes in type conformation throughout a lactation period, and averages such as the above may cover up some of those wide individual differences. A photographic record kept on many of the cows that were classified at various times throughout their lactation is valuable and brings out very clearly how greatly some cows change in appearance, not only with stage of lactation but also with age.

DISCUSSION

The results of the project thus far seem to indicate quite definitely that the variations in type ratings of individual Ayrshire cows are considerable over a period of years. These variations are due to several causes, among which are age, stage of lactation, degree of fleshing of the animal, and the differences in inspectors. However, from an analysis of the data, along with the criticisms made of each cow by each inspector at each classification, most of the wide variations in type ratings of individual cows apparently could be attributed to inability of inspectors to attach equal significance to certain faults of a cow. This seemed particularly true when the animals were criticized for defects in udder, feet, and legs. For example, 22 of the 26 cows that had a range of two or more grades changed at least two official grades in two consecutive classifications (an interval of 3 to 6 months). For 19 of these animals the main faults recorded were crooked legs, bad feet, or udders defective in either shape or attachment. As a general rule, the inspectors agreed very closely as to their appraisal of faults other than those found in the feet, legs, and udders.

These results indicate that uniform cuts for the more important defects in feet, legs, and udder should be adopted. Perhaps this can best be accomplished through a series of pictures of cows that exemplify pictorially the type and seriousness of the defects involved. Then perhaps definite point cuts could be determined for the various types of faulty legs, feet, and udders, and these cuts applied by each inspector.

Obviously, the standardizing of the human factor is still the most perplexing problem in type classification work, and consequently, by deliberately planning to use the largest possible number of inspectors, variations in the results were invited. Type rating technique has been and is being improved and, no doubt, will continue to improve. However, the results of this experiment are quite similar to those found by Johnson and Lush (2) in their study of type in Holstein-Friesians,—namely, that two or more type ratings of an individual cow are a far more accurate guide to her true type conformation than is one official rating which can never be lowered irrespective of future conformation changes.

SUMMARY

Eighty Ayrshire cows at the West Virginia Agricultural Experiment Station were rated for type an average of five times over a period of 4.5 years by nine different inspectors. A summary of these results shows that 54 animals or 67.5 per cent had a range of one grade or less, while 26 or 32.5 per cent had a range of two or three grades.

The relationships (repeatabilities) between ratings given a cow by the same inspector at different times were calculated for three different inspectors and found to be 0.73, 0.82, and 0.62, while the repeatability of ratings given the same cows by different inspectors was calculated to be 0.55.

Type ratings were found to be somewhat higher on older cows and, in addition, were higher on cows classified during the first 3 months of a lactation or the last 2 months as compared with the middle segment of the lactation.

It is concluded that several type ratings on an individual cow form a far more accurate guide to her true type conformation than does one official rating which can never be lowered.

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THE UTILIZATION OF LACTOSE BY THE DAIRY CALF FED NORMAL OR MODIFIED MILK DIETS¹

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Extensive studies with adult rats have shown that the addition of fat to skim milk diets markedly reduces the galactosuria observed when skim milk plus iron, copper, and manganese is fed as the sole diet (3, 8, 9). Little is known of the effect of fat on the utilization of lactose by the young calf or by other species. Results obtained on one calf and one pig (9) indicate that these animals perform in a manner similar to the rat. Both of these animals were found to utilize lactose completely when a whole milk diet was fed; however, after 2 weeks of skim milk feeding, considerable amounts of galactose were found in the urine. The galactose excretion apparently could be reduced to normal levels when 4 per cent corn oil was fed in addition to skim milk.

It was of importance, therefore, to extend these observations on the utilization of milk sugar by the young calf. The absorption and assimilation of lactose was studied when skim milk, whole milk, or modified whole milk diets were fed.

EXPERIMENTAL PROCEDURE

Ten male calves were used in these experiments, six Holsteins and four Jerseys. As these animals became available, they were fed either whole milk or skim milk diets. The group fed whole milk was composed of four calves, three Holsteins and one Jersey. Two of these animals received whole milk for the entire experimental period of 42 days, one calf was changed to a skim milk diet after 18 days, and the remaining calf was removed from the experiment after receiving whole milk for 12 days.

After the calves were taken from their dams, a daily milk allowance of 6 lb. for Jerseys and 8 lb. for Holsteins was pail-fed. The allowances were increased during the experiment according to the performance of the individual calf.

The group fed skim milk was composed of six calves, three Holsteins and three Jerseys. Two of these calves received a specially prepared low-fat colostrum for the first 4 days and skim milk thereafter; two suckled their dams for the first 24 hours; two received no colostrum and were fed skim milk from birth. Skim milk allowances were the same as for whole milk for the first 2 or 3 days, but were increased thereafter to compensate partially for the lower energy value of the skim milk.

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¹ Some of the data were taken from a thesis submitted by Jorge Rojas in partial fulfillment of the requirements for a Master of Science degree.

The skim milk diet was supplemented with 25,000 USP units of vitamin A per day by capsule as fish-liver oil and with approximately 1,000 units of vitamin D (viosterol). Since several investigators have shown that milk is low in iron, copper, manganese, and magnesium (1, 5, 6), the rations were supplemented with 7, 0.7, 0.6, and 43 mg., respectively, of these elements per pound of milk. Cases of scours or other digestive difficulties were treated successfully with 0.25 ounce of sulfaguanidine *per os* daily for 4 days.

All calves were kept in individual stalls with straw bedding, except during the days when urine collections were made. The urine was collected



FIG. 1. Apparatus used for making quantitative urine collections from young dairy calves. The apparatus is fastened firmly in order to avoid losses of urine when voided and can be adjusted according to the size of the animal. The animal is placed in a close-fitting elevated crate when urine collections are made, and the hose of the apparatus is passed through a hole in the bottom of the crate and fastened to a bottle placed under the crate.

for 24 hours at regular intervals. An apparatus and crate were designed to obtain quantitative urine collections. The apparatus used is shown in figure 1. The volume of urine voided was recorded and aliquots taken for sugar analyses.

Sugar determinations were made by the Shaffer-Hartmann method (10). The values for sugar were calculated by the use of the Munson-Walker tables (7) with the factor 1.22 to convert glucose values to galactose. It is recognized that urine contains reducing substances other than sugars which

TABLE I
Effect of feeding whole milk on the urinary excretion of galactose

Periods	Days	Whole milk fed (lb. per day)	No. of calves	No. of urine collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	Galactose excreted \times 100 Lactose ingested
I	1-6	7.3	5	6	363.0	5.580	3.4*
II	7-12	7.8	4	8	131.4	3.142	1.8
III	13-18	9.0	3	4	118.0	3.112	1.5
IV	19-24	8.2	2	4	107.8	2.448	1.3
V	25-30	8.5	2	4	118.1	3.071	1.6
VI	31-36	9.0	2	4	91.5	2.218	1.1
VII	37-42	8.1	2	4	140.1	3.433	1.9

*Holstein calf no. 1139 excreted 12.274 g. galactose during the first collection.

may influence the values obtained. The results are expressed as galactose in all cases, although its presence was not confirmed by osazone tests. The actual values, therefore, cannot be interpreted unequivocally as galactose, but they serve as a useful measurement for comparing the effects of different dietary regimens, as has been widely used by other investigators. The accuracy of the method as applied to urine was checked, and recovery values for galactose added to urine were satisfactory, all within the range of ± 5 per cent of the theoretical.

RESULTS AND DISCUSSION

Previous reports on the utilization of lactose by rats fed skim milk diets were checked. Adult rats fed a skim milk diet for 2 weeks excreted an

TABLE 2
Effect of feeding skim milk on the urinary excretion of galactose

Periods	Days	Skim milk fed (lb. per day)	No. of calves	No. of urine collections	Galactose per 100 ml. urine (mg.)	Total galactose excreted (g. per day)	Galactose excreted \times 100 Lactose ingested
I	1-6	8.2	5	9	182.8	3.030	1.6
II	7-12	9.5	6	12	134.9	2.609	1.3
III	13-18	10.4	6	12	106.6	2.906	1.3
IV	19-24	11.6	7	14	121.2	4.149	1.6
V	25-30	11.7	6	10	120.8	3.319	1.3
VI	31-36	12.1	6	10	132.0	4.680	1.7
VII	37-42	12.3	5	8	115.4	4.751	1.7

average of 20 per cent of the ingested galactose. Substituting whole milk for skim milk in the diet reduced the galactose excretion to 2 per cent of the intake. These results confirm those of other investigators (3, 8, 9).

Summaries of the results for the groups of calves fed whole milk and skim milk diets are shown in tables 1 and 2. These data are subdivided into 6-day periods, which show that the amounts excreted did not increase as the experiments progressed. These results do not agree with those of Schantz *et al.* (9). In no case were values found for galactose excretion that approached the figure of 16 per cent of the intake reported by these investigators. The data show that only a small percentage of the galactose ingested was excreted in the urine, regardless of the dietary treatment used. Furthermore, no difference was observed in the values for galactose excretion between calves maintained on whole milk or skim milk diets for periods up to 42 days.

An inspection of the data for each individual animal failed to reveal any appreciable differences from the data shown as averages for all animals within each group. In some instances, an animal would show a sudden rise in urinary galactose (from 1 to 4 per cent of the intake) but immediately the amount excreted dropped to what can be considered normal. The variation that occurred in urinary galactose from day to day for individual

TABLE 3

Effects of feeding additional lactose to calves receiving skim milk diets

Days on experiment	Ration per calf per day	Total galactose excreted (g. per day)	Galactose excreted $\times 100$ Lactose ingested	Health remarks
(Holstein calf no. 895)				
38-42	12 lb. skim milk	2.938*	1.1	Healthy appearance
44	12 lb. skim milk			
	320 g. lactose	24.832	4.2	Diarrhea
46	12 lb. skim milk			
	320 g. lactose	34.366	5.8	Diarrhea
48	12 lb. skim milk	9.706	3.6	Improved
51	12 lb. skim milk	9.034	3.3	Improved
(Jersey calf no. A42)				
32-39	10 lb. skim milk	6.653†	2.9	Healthy appearance
40	10 lb. skim milk			
	250 g. lactose	19.458	4.1	Diarrhea
42	10 lb. skim milk			
	250 g. lactose	36.017	7.6	Diarrhea
44	10 lb. skim milk	18.693	8.2	Improved
46	10 lb. skim milk	5.849	2.6	Improved
48	10 lb. skim milk	11.792	5.2	Improved

* Average for 2 collections.

† Average for 3 collections.

animals and between different animals can be explained at least in part by variations in the volume of urine voided. This variation was appreciably less when the values were calculated for 100 ml. of urine. The percentage excreted was not dependent upon the amount of milk consumed. The calf maintained on whole milk for 18 days and subsequently on skim milk for 24 days failed to show any difference in the per cent of lactose excreted as galactose for the two periods (an average of 1.9 per cent when whole milk was fed and 1.7 per cent for the period when skim milk was fed).

Another calf 2 months of age that had been receiving hay and grain and subsequently was fed only skim milk for 4 weeks did not show any increase in sugar excretion when the latter feeding regimen was used.

Several fecal samples were collected to determine whether the lactose was being absorbed effectively, particularly by calves that were scouring. No appreciable quantities of reducing substances were detected in these samples; therefore, it appears that the calves were assimilating the ingested sugar efficiently.

In later phases of the work, after two of the calves had been fed skim milk for 42 days, the lactose content of their ration was doubled by the addition of lactose to the skim milk in order to determine the effect of increasing the percentage of lactose ingested on its utilization by the calf. The results obtained with these calves are shown in table 3. Addition of lactose to the milk decreased the percentage of sugar utilized by the animal. One of the calves excreted approximately 16 per cent of the ingested galactose. This figure approaches that reported by Schantz *et al.* (9) with skim milk feeding. Within a few hours after the lactose-enriched milk was fed, both calves were afflicted with diarrhea. This symptom has been observed in rats receiving high-lactose diets (2, 4). Additional work on the effect of feeding equivalent amounts of other carbohydrates would be valuable in order to determine whether the rise in sugar excretion occurs only with the ingestion of lactose.

Other observations on the performance of the calves fed whole milk or skim milk diets were of interest. Calves fed the skim milk diets made slower gains, undoubtedly due largely to the reduced caloric intake. Cases of scours occurred in calves fed skim milk as well as those fed whole milk; however, with the latter, a milder form was encountered. Most cases of scours responded satisfactorily to treatment with sulfaguanidine.

The data obtained in these experiments show that the young calf up to 3 months of age does not excrete appreciable quantities of galactose in the urine when receiving either whole milk or skim milk diets. Apparently the absence of fat in the diet does not interfere with the utilization of milk sugar by the young calf.

The work with the rat has been conducted with older animals, and no observations are available on the ability of suckling rats to utilize lactose when fed skim milk diets. Present results with calves suggest that the very young animal, being adapted to the milk diet, utilizes lactose efficiently. Further studies are needed to determine the difference, if any, between the very young and the older bovine in the utilization of lactose.

SUMMARY

1. The effect of feeding whole milk or modified milk diets to dairy calves on the amount of sugar excreted in the urine was determined. Equipment was designed for quantitative urine collection.

2. Calves fed whole milk or skim milk for periods up to 42 days utilized the dietary lactose very effectively. No difference was observed in the galactose excretions for the two groups. The amount excreted, measured as galactose, ranged from 1.1 to 3.4 per cent of the total lactose ingested.

3. When the lactose content of the milk was doubled, the result was an increase in urinary galactose equivalent to 8 per cent of the lactose ingested. The galactosuria was accompanied by diarrhea and unthriftiness of the animals.

The authors are indebted to Dr. F. W. Hill, Western Condensing Company, Appleton, Wisconsin, for supplying some of the lactose used in this study; to Mead, Johnson and Co. for supplying the vitamin D concentrate; and F. E. Booth Company for supplying some of the vitamin A concentrate used in this study. Mr. F. W. Taylor assisted with the care of the animals as part of a collaborative research program on the utilization of vitamin A and carotene by calves fed whole milk or modified milk diets.

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THE RELATIONSHIP OF MAMMARY DEVELOPMENT AND BODY WEIGHT¹

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A method of selecting dairy calves on the basis of mammary development has been presented by Swett and Matthews (1). The extent of the mammary development of the heifer calf at 3 to 4 months of age has been demonstrated to be in direct relationship with the heifer's later productive ability (1).

This report deals with a study of the relationship of the mammary development to the body weight of Holstein and Guernsey heifer calves at 3 and 6 months of age.

EXPERIMENTAL PROCEDURE

Nineteen heifer calves (10 Holsteins and 9 Guernseys) were fed six grain rations which resulted in various rates of body growth. The original study involved an investigation into the possibilities of utilizing distillers dried solubles and distillers dried solubles with grains in the rations of dairy calves. While there were no statistically significant differences in the rates of growth of the several groups of calves, considerable variations existed in this respect between calves within the groups. The mammary development of all heifer calves was measured by the technique set forth by Swett and Matthews (1) at 3 and 6 months of age. Body weights were determined at the same ages.

Measurements were made of the width and length of each quarter at 3 months of age. Since the udders were in the half stage when the calves were 6 months of age, only the length of each half and the width of each quarter were measured at this age.

The mean width of the mammary tissue of the four quarters was correlated with body weight at 3 and 6 months. The mean length of the mammary tissue of the four quarters was correlated with body weight at 3 months. However, at 6 months it was necessary to use the mean length of

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TABLE 1

Summary of udder measurements of Holstein calves at 3 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
285	20.25	194	20.75	94
170	15.25	176	17.25	83
270	17.75	171	22.75	73
186	16.25	165	16.25	80
207	16.50	163	14.75	65
176	14.25	155	15.25	71
187	14.25	153	15.00	68
231	11.75	144	12.75	56
205	15.75	139	15.50	61
212	11.50	123	10.50	46

the mammary tissue of the two halves because the udder development had reached the half-stage. Similar correlations also were made with gain in body weight from 8 days to 3 and to 6 months.

EXPERIMENTAL RESULTS

The udder measurements and body weights of the ten Holstein calves at 3 months of age are summarized in table 1. These data are presented in order of body weights. The correlation coefficient between the average width of the quarters and body weight was $+0.844^{**2}$. A correlation coefficient of $+0.821^{**}$ was determined for the average length of quarters and body weight for these same ten calves. In table 2 are the data for nine Guernsey calves. A correlation coefficient of $+0.909^{**}$ was found for body weight and the average width of the quarters. For the average length of the quarters and body weight a correlation coefficient of $+0.917^{**}$ was

TABLE 2

Summary of udder measurements of Guernsey calves at 3 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of quarters	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
200	24.25	191	27.00	99
199	19.5	188	23.5	93
169	12.5	133	12.75	53
227	10.25	131	14.25	52
158	13.00	128	11.25	52
226	8.5	117	9.5	42
153	7.75	109	10.5	46
181	13.75	107	14.5	55
178	6.75	98	...	34

* The glandular tissue was too small to measure accurately.

² * = significant.

** = highly significant.

TABLE 3

Summary of udder measurements of Holstein calves at 6 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
207	46.5	396	77.0	298
270	45.25	378	82.0	280
186	41.25	371	53.0	286
187	31.5	349	67.0	264
231	45.75	340	70.0	252
205	47.5	337	93.0	259
285	41.5	334	69.5	234
170	40.5	333	73.5	240
176	33.75	319	70.5	235
220	51.5	309	75.0	210
212	41.5	273	86.5	196

found. The correlation coefficients were statistically significant for both length and width of the glandular tissue for the Holstein and Guernsey calves.

The summary of udder measurements and body weights for 11 Holstein calves at 6 months of age is presented in table 3. The correlation coefficient for the average width of the glandular tissue and body weight was found to be + 0.0836 and - 0.29 for the average length of the half stage of the udder and body weight. When tested statistically neither of these correlation coefficients was significant.

The summary of the measurements for eight Guernsey calves at 6 months of age is presented in table 4. The correlation coefficient for the average width of each quarter with body weight was + 0.444. However, this coefficient was not significant. The average length of the half stage of the udder and the body weight had a correlation coefficient of + 0.834*. A *t* value of 3.707 was necessary to be highly significant with six degrees of freedom. A value of 3.701 was obtained when the *t* value was determined. Thus the length of the udder and body weight were significantly related.

TABLE 4

Summary of udder measurements of Guernsey calves at 6 months of age

Calf no.	Av. width of quarters	Body weight	Av. length of halves	Gain in body weight
	(mm.)	(lb.)	(mm.)	(lb.)
200	41.5	357	95.0	265
226	53.5	293	89.0	218
227	36.0	286	73.0	207
153	31.75	281	63.5	218
158	31.0	275	60.5	199
169	37.25	274	64.0	194
178	31.25	272	72.0	208
181	32.75	266	58.5	214

Mammary development was correlated with rate of growth as determined by gain in body weight from 8 days to 3 months of age. A coefficient of $+0.812^{**}$ and $+0.7707^{**}$ was found for width and length, respectively, for the Holstein calves. Also, with the Guernsey calves, a correlation coefficient of $+0.9659^{**}$ was calculated for width and $+0.9756^{**}$ for length of the secretive tissue, and gain in body weight.

No significant relationship was shown to exist between gain in body weight from 8 days to 6 months and udder development at 6 months of age. Correlation coefficients of -0.006 for width and -0.2676 for length of mammary secretive tissue were determined with the Holstein calves, while correlation coefficients of $+0.345$ (width) and $+0.2386$ (length) were determined with the Guernsey calves.

SUMMARY

Nineteen calves of the Holstein and Guernsey breeds were used to determine the relationship of mammary development to body weight. A highly significant statistical relationship was found to exist between the development of the mammary secretive tissue and body weight of both the Holstein and Guernsey heifer calves at 3 months of age. Highly significant correlations also were found between mammary tissue development and gains in body weight from 8 days to 3 months of age of the heifer calves of both breeds.

The lengths of the secretive tissues of Guernsey calves were related significantly to body weight at 6 months of age; however, there was no correlation in this respect with the Holstein calves at this age. There was no significant correlation between the width of the mammary secretive tissue and body weight of either breed at 6 months of age. No significant correlation was found between the gains in body weight from 8 days to 6 months and mammary development of either breed.

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PERMANENCY OF SYNTHETIC ASCORBIC ACID ADDED TO MILK¹

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Hand (3) and others have shown that the reduced ascorbic acid content of raw, commercial cow's milk decreases rapidly during the first week after it is drawn from the cow. In his study, the average ascorbic acid value for 12 samples of milk was 19 mg. per l. at the beginning of storage at 1° C. and 7 mg. per l. 6 days later. Thus about 63 per cent of the original reduced ascorbic acid had disappeared from the milk during a 6-day storage period. Subsequently, Holmes and Jones (5) determined the loss of reduced ascorbic acid in mare's milk. They found that the rate of disappearance of ascorbic acid from mare's milk was only about one-seventh that reported by Hand for cow's milk. Since the composition of cow's milk and mare's milk is dissimilar in various respects, it is possible that a number of factors may influence the rate of loss of reduced ascorbic acid from the two types of milk. One obvious difference in composition is the amount of reduced ascorbic acid in the original milk. Hand reported that his samples of cow's milk contained from 14.8 to 22.8 mg. of ascorbic acid per l., whereas Holmes and Jones used samples of mare's milk that contained from 86 to 161 mg. of ascorbic acid per l. Accordingly, it was decided to determine the rate of loss of reduced ascorbic acid from cow's milk to which a sufficient amount of synthetic ascorbic acid had been added so that the ascorbic acid content of the milk approximated that of the mare's milk referred to above.

EXPERIMENTAL PROCEDURE

Since the stability of reduced ascorbic acid had been determined for raw mare's milk, raw cow's milk was used in this study. Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of synthetic ascorbic acid to a liter of milk. After the ascorbic acid was added, the milk was shaken thoroughly. One sample each of milk containing 75 mg. and 150 mg. of added ascorbic acid per l. was prepared per day. The enriched milk was placed in 500-cc. flasks and stored in the dark at 10° C. When the samples were prepared, the flasks were completely filled, but as aliquots were taken day by day for assay, the volume of milk decreased and the volume of atmosphere increased correspondingly. These conditions were the same as for the study of the stability of ascorbic acid in mare's milk and they were similar to the conditions in the average household where milk is stored in

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the refrigerator and at irregular intervals variable amounts are removed from the milk bottles.

The storage period was 10 days and only one sample of each series was placed in storage at a time. The amount of reduced ascorbic acid in all cases was determined by the method described by Holmes and Jones (5), and bentonite was used for clarification.

RESULTS AND DISCUSSION

The average values for the ascorbic acid assays of the two series of samples are reported in figure 1. The samples of the original milk before

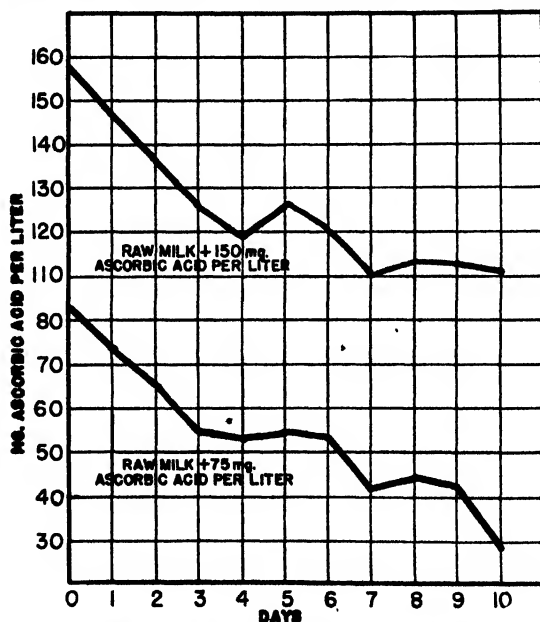


FIG. 1. Rate of loss of synthetic ascorbic acid from cow's milk.

the addition of the synthetic ascorbic acid contained, on an average, 9.5 mg. of reduced ascorbic acid per l. This value is in agreement with 7.5–9.2 mg. per l. reported by Christen and Virasoro (1), 10.8 mg. per l. by Lojander (9), and 12.2 mg. per l. by Mosonyi and Polónyi (10). However, these values for the ascorbic acid content of raw cow's milk definitely are less than those usually reported for milk—*i.e.*, 16.4 mg. per l. reported by Holmes *et al.* (7), 17.1 mg. per l. by Stewart and Sharp (12), 17.4 mg. per l. by Woessner *et al.* (13), 19.7 mg. per l. by Holmes *et al.* (6) and 22.2 mg. per l. by Sharp *et al.* (11).

When the two series of samples of ascorbic acid-enriched milk were placed in storage, their average reduced ascorbic acid contents were 83.0 mg.

and 157.5 mg. per l., respectively. Thus, losses of about 1.7 per cent and 1.2 per cent, respectively, occurred while the milk was being enriched and prepared for study. At this time the milk was exposed to laboratory temperature and full daylight but not to sunshine.

During the first 3 or 4 days of storage, reduced ascorbic acid was lost more rapidly and more consistently than during the remainder of the experimental period. For the series of samples of milk to which 75 mg. per l. of ascorbic acid was added, the loss of ascorbic acid was 34 per cent during the first 3 days or 11 per cent per day, and 33 per cent during the remaining 7 days or 5 per cent per day, with an average loss of 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid was added per l., the loss was 24 per cent for the first 4 days or 6 per cent per day, and 5 per cent for the next 6 days or 1 per cent per day, with an average loss of 3 per cent per day for the 10 days the milk was in storage. These losses are decidedly less than those reported by Gunsalus and Hand (2), who noted a reduction of reduced ascorbic acid of from 14.9 mg. to 1.7 mg. per l. or an average loss of 14.7 per cent per day during 6 days' storage of raw cow's milk. Hand (3) observed a loss of from 19.0 mg. to 7.1 mg. per l. of milk stored 6 days at 1° C., averaging over 10 per cent per day. Kothavalla and Gill (8) reported a loss of 26 per cent of ascorbic acid from cow's milk (Indian) stored at 45° F., or an average of over 8 per cent per day. Thus it appears from the data assembled here that when considerable amounts of synthetic ascorbic acid are added to raw cow's milk, the percentage of loss of ascorbic acid during storage is smaller than for the reduced ascorbic acid naturally occurring in raw cow's milk. It should be noted that, except for the period while the samples were being prepared at room temperature and for short intervals while the aliquots for assay were being withdrawn, the milk was stored in the dark at 10° C. Consequently, in this study as well as in the study of the stability of ascorbic acid in mare's milk, the effect of light and elevated temperatures upon the destruction of the ascorbic acid was kept at a minimum. Holmes and Jones (4) have shown that these factors cause exceedingly rapid destruction of reduced ascorbic acid in cow's milk. Obviously the data assembled here, together with those reported by the cited investigators, are not sufficient to provide a complete understanding of the factors and conditions that influence the rapid destruction of reduced ascorbic acid occurring naturally in cow's milk, or to provide means for preventing the unfortunate loss of this essential vitamin from one of the most valuable human foods.

SUMMARY

Two series of 20 samples each were prepared by adding 75 mg. or 150 mg. of ascorbic acid to a liter of raw cow's milk. The samples were stored in 500-cc. flasks in the dark at 10° C. As aliquots were removed day by

day for analysis, the volume of milk decreased and the volume of air in the flasks increased correspondingly. For the series of samples of milk to which 75 mg. of ascorbic acid per l. was added, the loss was 11 per cent per day for the first 3 days and 5 per cent per day for the remaining 7 days, or 7 per cent per day for the entire period. For the series of samples of milk to which 150 mg. of ascorbic acid per l. was added, the loss was 6 per cent per day for the first 4 days and 1 per cent per day for the remaining 6 days, or an average of 3 per cent per day for the 10 days of storage.

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SULFAMETHAZINE BLOOD AND MILK CONCENTRATIONS IN DAIRY COWS

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Bovine mastitis continues to be of great economic importance to all concerned with the production of milk. Reliable statistics on the losses attributed to the infection are unavailable, but it is considered to be the most important disease problem of the dairy industry (7). Various sulfonamides have been used in the treatment of mastitis. On the basis of present knowledge concerning the action of the sulfonamides, it would appear that the effectiveness of a sulfonamide depends upon adequate blood levels. The present study was initiated to determine the relationship between blood and milk concentrations of sulfamethazine.

CAUSES OF MASTITIS

Several species of bacteria may be associated with mastitis, but *Streptococcus agalactiae* has been recovered in the majority of cases. Staphylococci are considered to be second in importance, followed by other species of streptococci, coliform organisms, and corynebacteria (12). The exact manner of transmission of the disease is not known, but the most probable route of infection is through the teat duct. Environmental factors, repeated exposure to highly infective organisms, and injury to the udder, or a combination of factors, all have been held responsible for spread of the disease.

COMMONLY USED THERAPEUTIC AGENTS

Therapeutic agents have been relied upon to a great degree in the control of mastitis. While immediate infection can be corrected, re-infection cannot be prevented by these measures. However, correct herd management, in conjunction with good treatment procedures when disease does occur, will maintain a productive herd.

In vitro and *in vivo* studies have shown that sulfonamides are active against the streptococci, staphylococci, and other species of bacteria occurring in mastitis. In the early days of sulfonamide therapy, sulfanilamide was administered by mouth in the treatment of mastitis, and it continues to be

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used by many veterinarians. The reported results (10, 14) are conflicting. However, subsequent experience with sulfanilamide has shown its clinical use to be limited by its rather narrow range of bacteriostatic activity against those organisms other than streptococci which are pathogenic for man and animals.

It has been reported (3, 5, 13) that therapeutic agents for intramammary infusion, with the possible exception of penicillin in sterile water or physiological saline, frequently result in varying degrees of udder irritation, and, in some cases, cause permanent damage, as evidenced by decrease in milk flow and production of abnormal milk. Several investigators attempted to find an agent which would be effective in mastitis when administered parenterally, inasmuch as unsatisfactory results too frequently followed treatment by udder infusion. Because of the success of the therapeutic use of penicillin in human streptococci and staphylococci infections, and the successful use of intramammary infusions of penicillin in mastitis (1, 9, 11), work was undertaken to determine the permeability of the bovine mammary gland to penicillin parenterally administered. Such trials have been disappointing (2, 6, 15, 17), and it was found that penicillin was not present in the milk in detectable amounts or amounts sufficient to affect existing mastitis infection. In this work, however, dosages of penicillin used in cows were not sufficiently great, as judged by amounts needed to control human disease effectively. Watts and McLeod (17) reported the use of doses of 1,000,000 Oxford units, with no diffusion of penicillin in the milk. Obviously, larger dosages, and the frequent administration necessary, generally would be economically unsound.

Welsh *et al.* (18) showed that sulfamethazine, the dimethyl derivative of sulfadiazine, maintained the highest blood concentration of seven sulfonamides tested, over a 24-hour period on a fixed intake. It has been reported to be among the least toxic of the sulfonamides in therapeutic dosages, and its action against both Gram-negative and Gram-positive organisms frequently has been shown. Lately, evidence has been presented that sulfamethazine therapy alone, or in combination with penicillin (8, 16), can correct immediate infection and keep cows in the milking string.

EXPERIMENTAL PROCEDURE

Four normal cows were used in these experiments. To permit correct comparisons, the same cows were used in all three tests, with rest periods of 12 and 5 days, respectively, after each trial. Freedom from clinical mastitis at the time of the trial was determined on the basis of udder palpation and physical appearance of the milk by strip cup test.

The cows were maintained under conditions comparable to those of the average farm. Water was continuously available at automatic foun-

tains. Cows regularly were turned out to pasture. They were milked at 12-hour intervals, and, at the time of the trial, they were producing a minimum of 40 lb. of milk per day.

The dosage used throughout the experiment was 1.5 grains per lb. of body weight on the first day, and 1 grain per lb. of body weight on the second day. This dosage was administered in three ways: (a) One daily

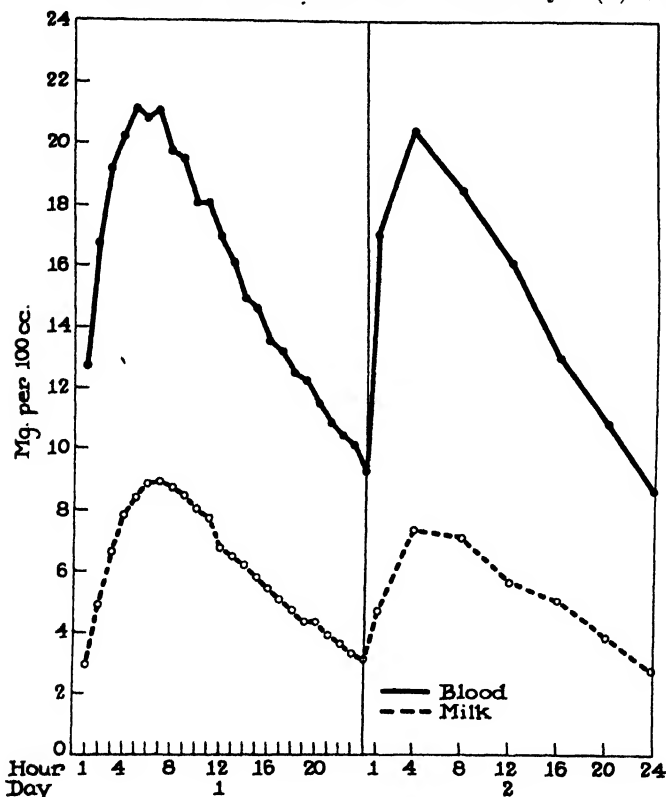


FIG. 1. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, subcutaneously; 2nd day, 1 gr./lb. body weight, subcutaneously. Milked at 0 and 12 hr.)

dose of sodium sulfamethazine 25 per cent w/v sterile solution was injected, subcutaneously, into each of four cows. (b) Sulfamethazine powder, in 1-ounce capsules, was administered orally, once a day, to two cows. (c) Sodium sulfamethazine 10 per cent w/v sterile solution was infused into the udders of four cows. Half of each total daily dose was administered immediately following complete morning and evening milkings, and equal amounts of each dose were infused into each quarter.

Sulfonamide analyses were made according to a modification (18) of the Bratton-Marshall method (4). Blood and milk samples were taken every hour for the first 24 hours, and at 1 hour, 4, 8, 12, 16, and 24 hours for the second 24 hours. Sulfamethazine determinations in milk were made for each period on each cow, using a composite sample from all quarters. Accurate records of milk production in pounds were kept for 9 days before the start of, as well as for the duration of, the experiment.

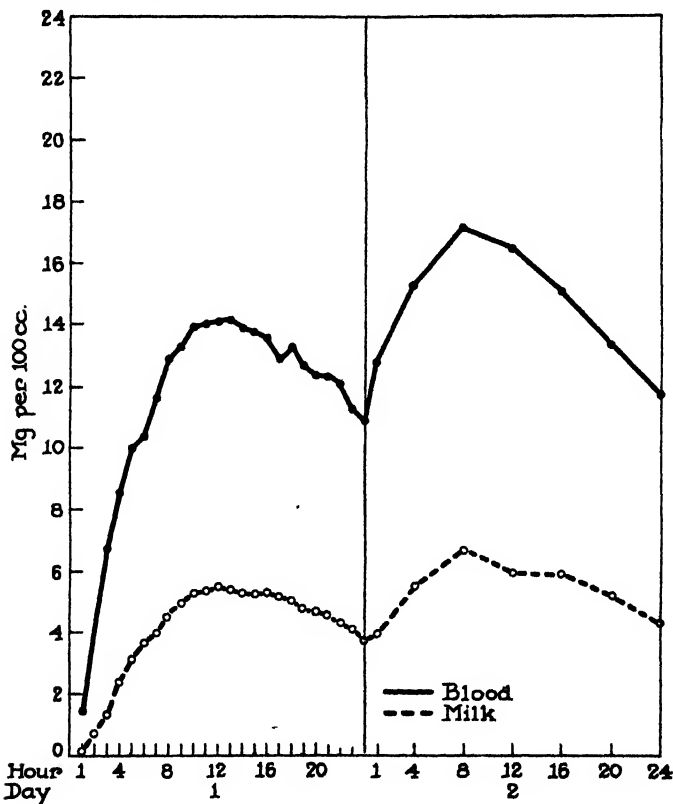


FIG. 2. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, orally; 2nd day, 1 gr./lb. body weight, orally. Milked at 0 and 12 hr.)

RESULTS

Average sulfamethazine blood and milk concentrations following subcutaneous administration are shown in figure 1. High blood levels were attained promptly, reaching a peak at about the fourth hour. The milk level curve closely followed the blood level curve, indicating that the

concentration in the milk is directly dependent on the blood concentration. The milk level was slightly less than half the blood level, which shows that an adequate milk level is dependent on a high blood level.

Figure 2 shows blood and milk concentrations after oral administration. The levels increased more slowly, reaching a peak on the first day between

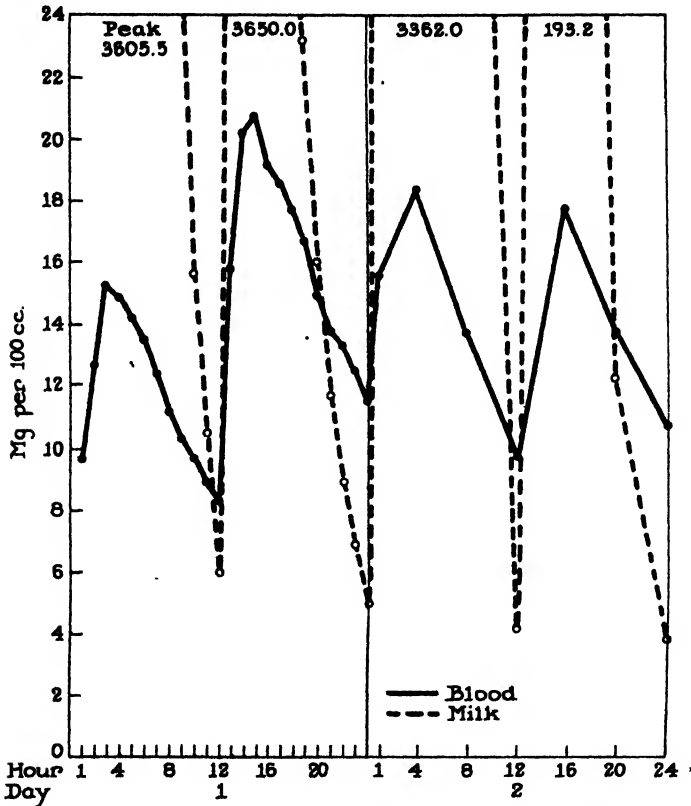


FIG. 3. Free sulfamethazine blood and milk concentrations in cows.

(Drug administration: 1st day, $1\frac{1}{2}$ gr./lb. body weight, intramammary infusion; 2nd day, 1 gr./lb. body weight, intramammary infusion. Half of each daily dose was administered immediately following the complete morning and evening milkings, and equal amounts of each dose were infused into each quarter. Milked at 0 and 12 hr.)

the eighth and twelfth hours and, on the second day, at about the eighth hour. The levels attained were not so high as after subcutaneous administration, but, likewise, did not decrease so rapidly. On the second day, the levels attained were higher than on the first day. This would indicate that, with an acutely ill animal, a prompt high blood level should be attained

by parenteral administration and maintained thereafter by oral dosing. Here, too, the milk level curve closely followed the blood level curve.

The concentrations attained by udder infusion are shown in figure 3. As might be expected, milk levels were extremely high after each infusion, rapidly decreasing from the second to the twelfth hour. Blood levels averaging between 10 and 15 mg. per 100 cc. of blood were attained within 3 hours after the first infusion, and gradually decreased to slightly more than 8 mg. per 100 cc. by 12 hours; they were maintained considerably higher after each of the next three infusions. It is evident, therefore, that sulfamethazine diffuses from udder to blood as well as from blood to milk.

Immediately following udder infusion, flakes were observed in the milk. This condition persisted for approximately 4 hours, when the milk again was normal in appearance.

In the normal cow, the decrease in milk production following administration of the drug by any of the routes described was not considered significant. The animals were being handled continuously during the 2-day trial periods, and this, in itself, would affect milk flow. During the three trials, average milk production decreased by 14 per cent, 16.5 per cent, and 18.5 per cent, respectively, from the average daily production during the 9 days preceding the experiment.

SUMMARY

1. Sulfamethazine was administered to cows parenterally, orally, and by infusion, and blood and milk determinations were made at frequent intervals after administration.

2. Sulfamethazine diffuses freely from blood to milk and from udder to blood.

3. Concentrations of 5 mg. or more of the drug per 100 cc. of milk throughout the day depend upon a persistently high concentration (more than 10 mg. per 100 cc.) of the drug in the blood.

4. All three methods of administration resulted in the attainment of blood and milk concentrations considered to be bacteriostatically effective.

5. No evidences of systemic toxicity of the drug were noted.

6. In this experiment, it has been shown that levels above 5 mg. per 100 cc. of milk can be achieved after oral or parenteral administration of the drug given once a day.

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THE UTILIZATION OF β -CAROTENE, VITAMIN A ALCOHOL, AND THE NATURAL ESTER OF VITAMIN A BY HOLSTEIN HEIFERS^{1,2,3}

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Vitamin A is believed to be absorbed from the digestive tract in the alcohol form (1). The question has arisen as to whether vitamin A is most efficiently utilized when ingested as the alcohol form, as the natural esters of vitamin A or as β -carotene. A study was conducted to determine the relative efficiency of utilization of β -carotene, vitamin A alcohol, and the natural ester of vitamin A.

EXPERIMENTAL PROCEDURE

Six Holstein heifers between the ages of 12 and 15 months were placed on a low-carotene ration consisting of oat straw fed *ad libitum* and 10 lb. of a concentrate mixture low in carotene. The animals were maintained on this ration until the blood plasma vitamin A decreased to 6–8 γ per 100 ml. of blood plasma. They then were grouped into three pairs based upon age, body weight, and blood plasma vitamin A concentration. The three pairs of animals then received in rotation each of the three sources of vitamin A for a period of 20 days at the rate of 100 USP units of vitamin A per kg. of body weight per day. After the first and second feeding periods, the animals again were depleted to 6–8 γ of vitamin A per 100 ml. of blood plasma before starting the subsequent supplementary feeding. Thus, after three feeding periods of 20 days each, all six animals had received the three forms of vitamin A. In all instances the vitamin A supplement was administered daily in capsules during the feeding periods.

Blood samples were taken at weekly intervals during the depletion periods and daily during the feeding periods, except in the last half of the first 20-day test period; during this time, they were taken every other day. Blood plasma carotene and vitamin A were determined using the methods of Moore (3) and Kimble (2), respectively, while using an Evelyn photo-electric colorimeter.

Body weights were determined twice a month during the depletion periods and every 3 days during the feeding periods.

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² The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

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⁴ Now associated with the University of Idaho, Moscow.

TABLE 1
The concentrations of blood plasma vitamin A of Holstein heifers fed β -carotene, vitamin A alcohol, and the natural esters of vitamin A^{a,b}

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Period of β -carotene administration																			
717	5	6	6	5	6	11	11	9	10	9	10	8	7	8	7	8	8	7	7	7
718	13	9	9	11	10	8	10	9	11	*	12	*	8	*	10	*	11	*	9	9
724	15	6	9	7	4	5	4	5	4	5	5	6	4	4	4	5	10	4	6	7
725	4	4	4	4	1	10	7	7	9	6	4	4	5	2	5	7	5	5	5	9
726	11	9	10	6	6	8	8	8	10	*	8	*	6	*	*	6	*	8	*	8
730	10	6	8	7	5	2	1	2	3	2	5	4	4	2	4	5	4	4	2	5
Av.	9.7	6.7	7.7	6.7	5.3	7.3	6.8	6.5	7.5	6.3	7.7	5.5	5.7	4.0	6.2	7.5	6.7	5.5	6.3	7.5
	Period of vitamin A alcohol administration																			
717	10	9	9	10	8	8	8	9	10	12	11	12	13	10	11	13	12	10	13	12
718	9	12	15	14	14	17	18	17	20	21	23	15	12	13	14	14	14	13	12	14
724	11	11	12	12	7	8	9	10	12	*	12	*	8	*	13	*	12	*	11	12
725	11	6	8	6	4	4	4	6	4	7	7	7	9	10	7	8	9	7	11	10
726	5	9	8	10	4	12	10	11	12	11	13	11	10	10	11	11	10	12	10	10
730	8	10	9	10	10	12	17	11	14	*	13	*	10	*	11	*	12	*	12	11
Av.	9.0	9.5	10.2	10.3	7.8	10.2	11.0	10.6	12.0	12.8	13.2	11.3	10.3	10.8	11.2	11.5	11.5	10.5	11.5	11.5
	Period of administration of the natural esters of vitamin A																			
717	11	14	20	11	7	8	9	11	11	*	12	*	9	*	15	*	15	*	15	16
718	12	11	11	13	11	11	12	11	11	13	13	13	12	11	11	13	12	11	13	14
724	8	10	9	9	7	12	12	11	16	18	13	16	10	11	10	13	11	11	11	13
725	10	9	11	8	8	9	12	10	12	*	11	*	10	*	12	*	13	*	10	10
726	8	7	10	9	7	6	8	7	10	9	10	10	10	8	10	12	9	10	9	9
730	4	7	7	9	8	10	9	11	13	14	14	10	11	10	12	12	12	10	13	11
Av.	8.8	9.7	11.3	9.8	8.0	9.3	10.3	10.2	12.2	13.5	12.2	10.3	10.3	10.0	11.3	12.5	12.0	10.5	11.8	12.2

^a Fed at the rate of 100 USP units per kilo of body weight per day.

^b Expressed as γ of vitamin A per 100 ml. of blood plasma.

* No analysis.

TABLE 2
The blood plasma concentrations of carotene of heifers fed β -carotene, vitamin A alcohol and the natural esters of vitamin A^{a,b}

Heifer no.	Days of administration																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Period of β -carotene administration																			
717	52	58	56	58	69	67	69	69	82	80	91	94	89	85	85	100	103	94	85	98
718	40	44	46	50	52	50	52	48	48	*	61	*	67	*	78	*	78	*	73	78
724	63	65	76	65	69	80	85	80	76	80	85	91	94	96	96	94	96	96	96	98
725	33	35	37	42	44	50	50	52	58	63	59	61	58	56	59	63	56	61	61	63
726	50	54	58	54	58	56	69	65	65	*	86	*	96	*	86	*	86	*	89	87
730	42	44	54	52	56	58	58	54	54	58	63	65	61	61	63	65	65	65	59	61
Av.	46.7	50.0	54.5	53.5	58.0	60.2	63.8	61.3	63.8	70.3	74.2	77.8	77.5	74.5	77.8	80.5	80.7	79.0	77.2	80.8
	Period of administration of vitamin A alcohol																			
717	58	54	54	52	54	54	50	48	44	46	44	50	42	38	42	50	46	42	42	40
718	38	46	42	40	42	40	38	40	40	44	44	37	37	35	35	40	42	40	33	35
724	67	69	65	69	56	54	44	50	46	*	49	*	52	*	56	*	53	*	52	56
725	40	38	42	38	38	35	38	34	37	38	38	40	44	44	38	42	35	37	35	31
726	23	25	25	29	27	23	23	25	27	27	31	29	25	27	27	31	31	33	31	31
730	50	54	50	56	54	50	56	50	52	*	59	*	65	*	56	*	54	*	56	49
Av.	46.0	47.7	46.3	47.3	45.2	42.7	41.5	41.8	41.0	38.8	44.2	39.0	44.2	36.0	42.3	40.8	43.5	38.0	41.5	40.3
	Period of administration of the natural esters of vitamin A																			
717	76	78	78	74	78	65	61	67	65	*	70	*	68	*	76	*	75	*	66	67
718	48	54	48	50	48	44	44	44	42	48	46	52	52	46	50	50	50	48	48	44
724	67	67	63	63	67	61	59	58	61	69	56	65	54	54	52	59	54	54	58	59
725	42	48	42	42	38	37	35	33	*	43	*	43	*	48	*	42	*	46	*	42
726	50	50	56	50	48	48	46	40	44	48	50	48	48	46	46	46	46	46	42	38
730	33	33	31	29	29	27	27	27	26	31	31	27	25	38	25	27	29	27	31	29
Av.	52.7	55.0	53.0	51.3	51.3	47.0	45.3	45.2	45.7	49.0	49.3	48.0	49.2	46.0	48.5	45.5	50.0	43.8	47.8	46.0

^a Fed at the rate of 100 USP units per kilo of body weight per day.

^b Expressed as γ of carotene per 100 ml.

* No analyses made on first trial on these days.

RESULTS

Vitamin A values of 6 to 8 γ per 100 ml. of blood plasma appeared to be the critical level for these animals. When the concentration of vitamin A approached this level, the test animals stopped gaining in body weight. However, the animals usually resumed growth after about 10 days of supplemental feeding. The average time required to deplete the animals was 105 days following winter feeding, 24 days between the first and second feeding periods, and 30 days between the second and third feeding periods.

The data obtained relative to the analyses for vitamin A and carotene on the several trials are presented in tables 1 and 2.

In evaluating the blood plasma vitamin A data for the three supplementary treatments by an analysis of variance (table 3), a highly significant

TABLE 3
Analysis of variance of blood plasma vitamin A data

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total	179	2261	
Treatments	2	593	296.50 ^a
Individuals	5	393	78.60 ^b
Days	9	189	21.00 ^a
Interactions:			
Treatments \times individuals	10	242	24.20 ^a
Days \times individuals	45	137	3.04
Days \times treatments	18	134	7.44
Sampling error	90	573	6.37

^a Significant at the 1% level.

^b Approached significance at the 5% level.

difference was found between the treatments and between days of supplementation. The difference between heifers approached significance. On the basis of the least significant mean difference, it was determined that there was no significant difference in the blood plasma vitamin A concentrations of the heifers during administration of vitamin A alcohol or the natural esters of vitamin A. Both, however, produced a higher level (highly significant) of blood plasma vitamin A than did β -carotene. The following mean concentrations of blood plasma vitamin A (γ per 100 ml.) were found during the feeding periods: β -carotene feeding = 6.95, vitamin A alcohol feeding = 10.77, and the natural ester of vitamin A feeding = 10.83. The least significant mean difference at the 1 per cent level was 2.85.

In an analysis of variance of the determinations of blood plasma carotene of the heifers for the three treatments (table 4), a highly significant difference was found between treatments and between days on supplementation, but no significant difference was found between heifers. The following mean concentrations of blood plasma carotene (γ per 100 ml.) were determined

TABLE 4
Analysis of variance of blood plasma carotene data

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total	179	54,826	
Treatments	2	18,607	9,303.50 ^a
Individuals	5	13,997	2,799.40
Days	9	1,909	212.11 ^a
Interactions:			
Treatments × individuals	10	10,837	1,083.70 ^a
Days × individuals	45	839	18.64
Days × treatments	18	6,089	338.28 ^a
Sampling error	90	2,548	28.31

^a Significant at the 1% level.

during the feeding periods: β -carotene administration = 67.42, vitamin A alcohol administration = 43.57, and the natural ester of vitamin A = 49.28. The least significant mean difference at the 1 per cent level was 19.05. On this basis it was found that the feeding of β -carotene increased the blood plasma carotene concentration above (highly significant) that of the heifers receiving vitamin A. There was no significant difference in the blood plasma carotene concentrations when vitamin A alcohol or the natural ester of vitamin A was fed.

Linear regression lines were calculated for blood plasma vitamin A (Fig. 1) and carotene (Fig. 2) concentrations during the feeding periods. The regression equation for blood plasma vitamin A concentration when β -carotene was fed was $E = 7.422 - 0.0692 X \pm 0.0442$ (not significant); when the natural ester of vitamin A was fed, $E = 9.403 + 0.1412 X \pm 0.0384$

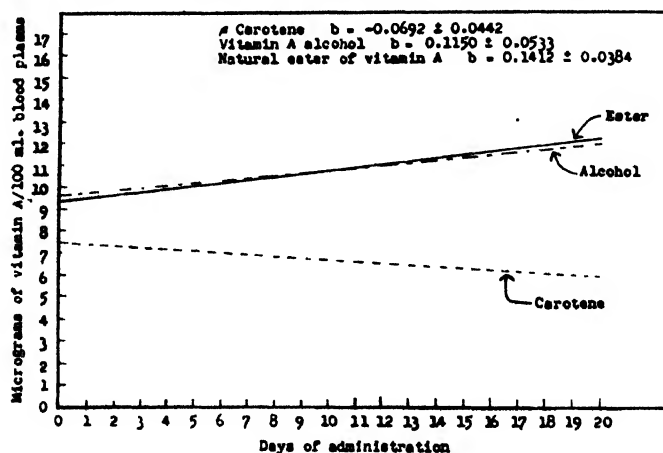


Fig. 1. The effect of the source of vitamin A upon blood plasma vitamin A levels.

(highly significant); and when the vitamin A alcohol was fed, $E = 9.61 - 0.1150 X \pm 0.0533$ (significant). Therefore, there was a significant increase in blood plasma vitamin A concentration when vitamin A alcohol or the natural esters of vitamin A were fed, and there was a slight decrease when β -carotene was fed. This decrease, however, was not significant.

Regression equations for blood plasma carotene concentrations are as follows: when β -carotene was fed, $E = 48.70 + 1.8325 X \pm 0.2186$ (highly significant); when the natural ester of vitamin A was fed, $E = 51.62 - 0.2934 X \pm 0.2211$; and when vitamin A alcohol was fed, $E = 46.45 - 0.3313 X \pm$

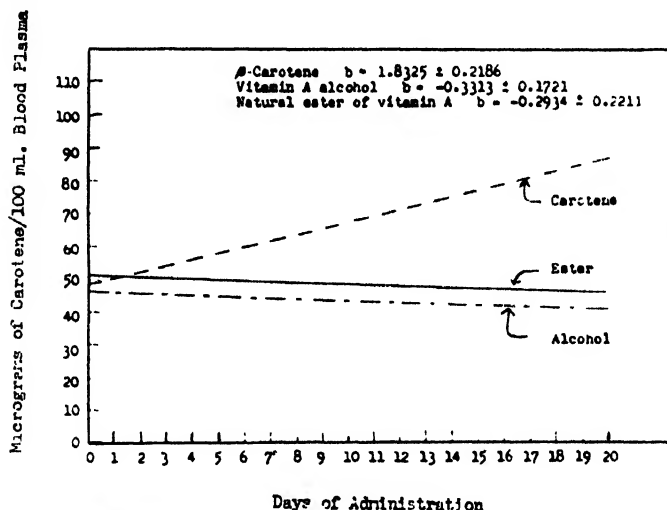


FIG. 2. The effect of the source of vitamin A upon blood plasma carotene levels.

0.1721. Therefore, feeding β -carotene increased the blood plasma carotene concentration significantly, but, when either of the two forms of vitamin A was fed, the blood plasma carotene concentration decreased slightly. This decrease, however, was not statistically significant.

CONCLUSIONS

1. The critical blood plasma vitamin A concentration was found to be 6 to 8 γ per 100 ml. blood plasma for Holstein heifers when gains in body weight were used as the criterion.

2. There was no significant difference in the efficiency of utilization of vitamin A alcohol and the natural esters of vitamin A by Holstein heifers when the blood plasma concentration of vitamin A was used as a criterion. However, both forms of vitamin A were utilized more efficiently than β -carotene.

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LIVEWEIGHT AND MILK-ENERGY YIELD AT VARIOUS FEEDING INTENSITIES¹

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The data used in the present paper are extracted from the records of the Input-Output experiment conducted by the United States Bureau of Dairy Industry in cooperation with the Agricultural Experiment Stations of Delaware, Maryland, Mississippi, New York (Geneva), Pennsylvania, South Dakota, Indiana, Michigan, New Jersey, and Virginia. In the last four of these Stations, pasture was used, thereby upsetting the determination of digestible nutrients (D.N.) intake. A primary objective in the use made of the Bureau data at the Illinois Station is to allocate D.N. intake between maintenance and lactation by the procedure of fitting a suitable equation, for which purpose only the first six of the above-mentioned stations provide adequate data. Pursuit of the primary objective has provided, somewhat incidentally, valuable material on the relation between liveweight and milk-energy yield, which is reported in the present paper.

A detailed account of the Input-Output investigation has been published by Jensen *et al.* (2).

PROCEDURE

The first 35 full calendar weeks of each lactation are used in the present study. Lactations which do not provide such a period are not used. That is, the present paper deals with partial lactations, starting within 9 days after calving (the first 2 days after calving being rejected in the original records) and continuing through the following 35 calendar weeks. The records provide a total of 255 such partial lactations.

Each 35-week partial lactation is extracted in seven subperiods of 5 weeks each, and the sum of seven subperiods represents the 35-week partial lactation. For each lactation the following items, among others, are calculated:

$D.N.$ = digestible nutrient intake for 35-week period, lb./day

FCM_s = milk-energy yield for 35-week period, lb. 4 per cent milk/day

W = average liveweight for 35-week period, lb.

W_1 = average liveweight for first 5-week subperiod, lb.

Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey breeds are repre-

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¹ The authors are indebted to O. E. Reed, Chief of the Bureau of Dairy Industry, for administrative approval of the present use of the records of the Input-Output investigation; also, to T. E. Woodward, Bureau of Dairy Industry, for painstaking care in providing photostatic copies of the weekly records maintained by the Bureau in conduct of the original investigation.

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sented in the present material (table 1). Perhaps the cows represent a cross section of dairy cows in American Experiment Stations.

RESULTS

Feeding intensity. Feeding intensity is calculated as $(D.N. - 0.008W)/FCM_s$, i.e., lb. D.N. for lactation per lb. FCM_s , allowing 8 lb. D.N. per day per 1,000 lb. liveweight for maintenance, in accord with the Haecker standard. The data are divided into feeding intensity classes as indicated in table 2. These classes are based on the actual rather than the intended feed intake. Consequently, the classes do not correspond strictly with the classes used by Jensen *et al.* (2), and there is the further important difference that the present analysis is based on a 35-week partial lactation, in contrast to a calendar-year record regardless of lactation status.

TABLE 1
The number of lactations and mean values by breeds

Breed	n	% Haecker	% Grain ^a	W_1	1000 FCM_s/W_1
Ayrshire	29	120	35	1035	25.7
Brown Swiss	25	107	52	1228	31.8
Guernsey	21	114	31	998	24.6
Holstein	104	114	46	1204	31.1
Jersey	76	123	45	844	31.0

^a Per cent of digestible nutrient intake supplied by concentrates.

Liveweight and yield. It previously has been proposed that cows possess a certain inherent tendency to produce milk under conditions of the commercial dairy, a lactational drive which may be quantitatively measured as FCM_s/W_1 . The immediate purpose of the present study is to see how FCM_s/W_1 is affected by feeding intensity.

In the light of previous work the postulate is advanced that FCM_s/W_1 fluctuates, as between cows, independently of W_1 . A proper test of this postulate is to fit, by least squares, the equation, $FCM_s/W_1 = a + bW_1$ and find the value of b and its standard error. For the 255 lactations as a body, as shown in the last line and last column of table 2, b is even smaller than its standard error. Such a value of b readily could arise by chance if its true value is zero. Hence, the postulate is valid so far as this particular body of observations indicates.

By a similar equational procedure the value of b (in terms of 100,000 FCM_s/W_1) within feeding intensity class works out to be 0.21 ± 0.23 or essentially the same result as that in total.³

If it is desired to use the power equation $FCM_s = aW_1^b$, the exponent b is 0.92 in total and 0.93 within feeding-intensity class. The exponent is derived from the means and linear regression. For example, in total, the ex-

³ In similar manner: within Station, $b = -0.109 \pm 0.036$; within breed, $b = -0.109 \pm 0.039$; within times milked daily (2 or 3), $b = -0.061 \pm 0.024$.

LIVEWEIGHT AND MILK-ENERGY YIELD

TABLE 2
Feeding intensity and milk-energy yield per unit liveweight

Class	Feeding intensity				Lactations	Mean		Value of <i>b</i> in the equation		
	Class limit	Mean	% Haacker	% grains ^a		<i>W</i> ₁	$\frac{1000}{FCM_e/W_1}$	$FCM_e = aW_1^b$	$FCM_e/W_1 = a + bW_1$	100000
1	< 0.30	0.273	83	28	35	1074	26.2	+ 1.34	+ 0.83 ± 0.67	
2	0.30	0.327	100	36	59	1078	27.3	+ 1.04	+ 0.10 ± 0.43	
3	0.35	0.374	114	47	71	1088	32.0	+ 0.99	- 0.04 ± 0.50	
4	0.40	0.422	129	52	54	1047	32.8	+ 0.95	- 0.17 ± 0.45	
5	0.45	0.474	145	53	20	1031	28.6	+ 0.92	- 0.21 ± 0.94	
6	0.50	0.600	183	53	16	968	30.3	- 0.07	- 3.34 ± 0.96	
All		0.381	117	44	255	1063	30.0	+ 0.92	- 0.23 ± 0.25	

^a Per cent of digestible nutrient intake supplied by concentrates.

ponent = $1 + (-0.22 \times 1063/3000) = 1 - 0.08 = 0.92$. This is a valid approximation for the present material.

While the above procedure indicates no difference in total and within feeding-intensity class, it does not necessarily follow that feeding intensity is without influence on the relation of liveweight to milk-energy yield. Table 2 shows the weight-yield relation for each of the six classes separately. None of the b 's is significant except the one for the 16 lactations of class 6. The W_1 distribution in class 6 is erratic and apparently the cows were more ravenous than representative.

A noteworthy feature is the rather consistent decrease in b as feeding intensity increases. The power-equation b shows this clearly. The data seem to suggest that FCM_s tends to be proportional to W_1 under customary feeding intensity (100 per cent or 114 per cent of Haecker standard for the lactation fraction). W_1 appears still more influential on FCM_s for under-feeding, which may trace back to the influence of fatness at calving.

DISCUSSION

The average of 255 35-week partial lactations (table 2) is 30.0 lb. of 4 per cent milk per day per 1,000 lb. W_1 . Davis *et al.* (1) report for the Nebraska Station dairy herd average values for 1000 FCM_s/W_1 of 33.3 for 131 Ayrshire lactations, of 30.6 for 77 Guernsey lactations, of 39.1 for 367 Holstein lactations and of 34.5 for 171 Jersey lactations. Feeding intensity for the Nebraska data is not recorded but no doubt is above 100 per cent Haecker for all lactations. Control of feeding intensity was a major point in the Input-Output experiment. It is presumed this control does not bias the results as between breeds.

SUMMARY

The relation of liveweight in pounds within 5 weeks after calving, W_1 , to milk-energy yield for the 35-week partial lactation in pounds of 4 per cent milk per day, FCM_s , is investigated by adjusting the equation, $FCM_s/W_1 = a + bW_1$, to observations from the Input-Output experiment of the Bureau of Dairy Industry. For all lactations (255), b is not significantly different from zero. The postulate that FCM_s tends to be proportional to W_1 is valid so far as indicated by this body of observations on five breeds of dairy cows taken as a whole. Essentially the same relation holds for each feeding-intensity class (83 to 183 per cent of Haecker). However, as between feeding-intensity classes, there is a consistent tendency for b to decrease as feeding intensity increases. The consistency of this tendency may give it some meaning.

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THE CHEMICAL COMPOSITION OF THE CRYSTALLINE DEPOSIT IN EVAPORATED MILK

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A white, crystalline deposit occasionally forms in evaporated milk during storage. It is found chiefly on the interior can surfaces but as the crystals increase in size and weight, agitation of the contents of the can gradually causes them to settle. The deposit does not appear in some samples of milk and, even when it is present, it remains unnoticed by most consumers. While the crystals are not harmful, they are at times a source of annoyance, especially in infant feeding when they obstruct the holes in nipples. Occasionally the crystal aggregates are mistaken for foreign particles.

The deposit cannot be redissolved in the milk after it has formed. The particles themselves vary from microscopic size to crystal aggregates $\frac{3}{8}$ -inch in diameter. They are dense and hard and should not be confused with the insoluble protein deposit which sometimes is found in evaporated milk.

Sato was the first to investigate the salt crystals of concentrated milk. On the basis of his determinations of calcium, magnesium, and phosphorus in salt crystals found in sweetened condensed milk (9, 11), he reported calcium citrate to be the chief constituent of these crystals. Only qualitative determinations for citrates were reported. Crystals of tyrosin, leucin, and cysteine also were found. Later Sato (10) reported on the examination of the sediment obtained from one 2-year-old can of evaporated milk. A quantitative analysis of this deposit was stated to show that it contained tricalcium and trimagnesium phosphates and tricalcium citrate. The quantitative data and the relative proportions of these salts in the evaporated milk were not given.

Mojonnier and Troy (7) condensed unheated skim milk over sulfuric acid to one-third its volume, stored it at 85° F. for 3 months, and then found a considerable quantity of calcium citrate had crystallized in the milk.

While this manuscript was in preparation, Gould and Leininger (3) published the results of quantitative determinations made in duplicate on a composite sample of crystals separated from evaporated milk. They found that the crystals were largely composed of calcium citrate.

This study of the crystalline deposit that forms in evaporated milk was conducted to determine quantitatively the composition of the crystals and to define the conditions that favor and retard their formation. This paper is concerned with quantitative determinations.

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EXPERIMENTAL PROCEDURE

Crystals were obtained from several hundred cans of commercial evaporated milk. The sediment from each can was washed with 25 per cent alcohol, thoroughly agitated, and decanted a number of times until the wash alcohol showed no turbidity. The crystals then were assumed to be reasonably free from foreign matter. They were air dried at room temperature, ground in a mortar, redried at a low temperature under vacuum, and preserved in glass-stoppered bottles for analysis.

The method of McCrudden (4, 5) was used for the calcium determinations. The calcium oxalate precipitate was ignited and weighed as calcium oxide. Magnesium (4, 5) was determined on the filtrate from the calcium analyses. Nitric acid was added to this filtrate and the solution evaporated to dryness to expel the ammonium salts. The residue then was dissolved in a little hydrochloric acid. The usual procedure was carried out and the precipitate was weighed as magnesium pyrophosphate.

The official gravimetric method (1) was used for the determination of phosphorus.

Citric acid was determined by the pentabromoacetone method as modified by Deysher and Holm (2), with a few additional changes. The procedure is outlined here in some detail because citric acid determinations often have been found difficult to make and because good results were obtained with this modification.

Five-tenths gram of the material is dissolved in 40 ml. of sulfuric acid (1 to 1 by volume) in a 250-ml. volumetric flask and several milliliters of 10 per cent phosphotungstic acid are added to precipitate the small amount of protein material. The contents of the flask are made up to 250 ml., thoroughly shaken, and filtered. Fifty milliliters, which is equivalent to 0.1 g. of the sample, are taken for the determination. Five milliliters of 37.5 per cent potassium bromide are added, followed by 5 per cent potassium permanganate added dropwise until a brown precipitate remains for at least an hour. The mixture then is placed in a refrigerator over night, after which the excess potassium permanganate is discharged with 20 per cent ferrous sulfate. After filtering, drying in the vacuum desiccator, and weighing, the precipitate is dissolved with alcohol and ether. The crucible again is dried and weighed. The loss in weight represents the pentabromoacetone which, multiplied by 0.424, is equal to anhydrous citric acid.

This method was tried on C.P. calcium citrate in which the water of crystallization had been determined. The recovery on four determinations was 98.7, 101.7, 100.3, and 99.4 per cent.

RESULTS

The analytical results obtained on four groups of crystals gathered from cans of commercial evaporated milk are given in table 1.

TABLE 1
*Analysis of crystals from 4 brands of evaporated milk**

Milk no.	Loss on ignition	CaO	MgO	P ₂ O ₅	Anhydrous citric acid	Loss on ignition + CaO + MgO + P ₂ O ₅
	(%)	(%)	(%)	(%)	(%)	(%)
1b	70.79	29.0	0.23	0.19	64.25 ^c	100.21
2b	70.40	29.22	0.12	0.44	63.97	100.18
3	65.80	29.90	0.12	4.43	60.71	100.25
4b	70.95	29.10	0.31	0.19	63.60 ^c	100.55

* Each group of crystals was collected from cans of milk produced in a single plant and processed on the same or on consecutive days.

^b Only a trace (0.02%) of SiO₂ was found in a composite of these samples.

^c Triplicate determinations; all others made in duplicate.

The calcium oxide was found to be about 29.0 per cent and the anhydrous citric acid 63.0 per cent. The composition of C.P. calcium citrate in terms of calcium oxide and anhydrous citric acid is 29.48 per cent and 67.33 per cent, respectively (6).

There was a 9 to 10 per cent loss in weight of the evaporated milk crystals at 120° C., which indicates that water of crystallization was present. According to Merck's Index (6) all the water of crystallization of calcium citrate is lost at 120° C.

The amounts of magnesium oxide in the different samples were fairly uniform but the quantity of phosphorus pentoxide in no. 3 was in large excess over the phosphorus pentoxide in the other three samples. This indicates the presence of a substantial quantity of calcium phosphate in no. 3.

Since the calcium in the calcium caseinate-calcium phosphate complex (8) of milk exists as tribasic phosphate, calculations were made to determine the quantities of tribasic magnesium and calcium phosphate and of calcium citrate that might be present in the crystals. Results of the calculations are presented in table 2. All the magnesium oxide was converted to trimagnesium phosphate, but this required more phosphorus pentoxide than was present in samples 1 and 4, giving the latter a negative phosphate balance.

TABLE 2

Calculated values^a for calcium and magnesium phosphates and for calcium citrate in the salt crystals of evaporated milk

Milk no.	MgO as Mg ₃ (PO ₄) ₂	P ₂ O ₅ balance	Remaining P ₂ O ₅ as Ca ₃ (PO ₄) ₂	CaO balance	Remaining CaO as CaCit. 4H ₂ O	Total Mg ₃ (PO ₄) ₂ + Ca ₃ (PO ₄) ₂ + CaCit. 4H ₂ O
	(%)	(%)	(%)	(%)	(%)	(%)
1	0.50	- 0.08		29.00	98.33	98.83
2	0.26	+ 0.30	0.65	28.87	97.90	98.81
3	0.26	+ 4.29	9.37	24.82	84.18	93.81
4	0.67	- 0.17		29.10	98.68	99.35

^a Calculated from the determined values shown in table 1.

The remaining phosphorus pentoxide in samples 2 and 3 was converted to tricalcium phosphate. The calcium balance remaining after the phosphorus pentoxide was exhausted was converted to calcium citrate. This required a little more citrate than was found in the samples. The sums of these calculated values for the phosphates and citrates are given in the last column of table 2. With the exception of sample 3, they are not far from 100 per cent.

There are other salt combinations that might be assumed to exist for the purpose of calculating the approximate composition of the crystals. However, in the absence of accurate data on the salt crystal structures, any determination of the manner in which the various components are combined must be deferred.

SUMMARY

Four groups of salt crystals that had separated from evaporated milk during storage were analyzed for calcium oxide, magnesium oxide, phosphorus pentoxide and citric acid. One group of crystals was high enough in phosphorus pentoxide to indicate the presence of almost 10 per cent tricalcium phosphate. Most of the crystals contained about 98 per cent calcium citrate and small but varying amounts of tricalcium and trimagnesium phosphates.

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THE ISOLATION AND PROPERTIES OF THE IMMUNE PROTEINS OF BOVINE MILK AND COLOSTRUM AND THEIR ROLE IN IMMUNITY: A REVIEW

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In mammalian life, the mother supplies the fetus and the newborn offspring with all of the substances necessary for growth. However, in addition to providing the usual food factors, the offspring is given the antibodies necessary to resist infectious diseases. Since the work of Ehrlich (8), it has been known that antibodies may be transmitted through the colostrum or first milk, passively immunizing the offspring by means of immune bodies which are ingested by mouth and then pass from the digestive tract to the blood stream. In addition, Ehrlich discovered that, in some species, immune bodies also may be transmitted through the placenta directly from the blood stream of the mother to the circulation of the fetus.

In the ruminants, placental transmission does not occur, and the colostrum is the sole source of antibodies for the newborn animal (9, 13, 22). Some years ago, Smith and Little (32, 33) investigated the factors concerned with the survival of newborn calves and found that intestinal infections were among the major causes of death. Feeding of colostrum was found to decrease the mortality enormously. Obviously, the antibodies transmitted by the colostrum are of great importance in enabling the newborn animal to resist infection. At about the same time, Howe (10) and Orcutt and Howe (17) observed that, after the ingestion of colostrum, agglutinins appear in the calf serum associated with a globulin which is precipitable at low concentrations of sodium sulfate. Other investigators (11, 21) have since found by electrophoretic analysis that the serum of the newborn calf does not possess any γ -globulin and that the appearance of slow-moving globulin occurs only after the ingestion of colostrum.

In recent years it has been amply demonstrated by many investigators that antibodies are associated with globulin components of the serum, and much has been learned regarding their properties (12). Until recently, no attempts were made to isolate and study the proteins associated with immunity from milk or colostrum.

The immune proteins of bovine colostrum and milk have been isolated in order to determine their relationship to the immune proteins found in blood serum (24, 25, 26). It obviously is of some importance to ascertain

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whether the immune bodies in the colostrum and milk are identical or similar to those in the maternal blood stream. It also is of interest to determine what happens to the colostrum antibodies during their passage into the blood stream of the newborn animal. The cow represents a good species for such a study because, in addition to the availability of milk and colostrum, the situation is not complicated by placental transmission of antibodies. It is convenient to refer to the colostrum and milk globulins which are associated with immunity as "immune lactoglobulins", although it is realized that the actual antibody content may account for only a very small portion of these fractions.

BOVINE IMMUNE PROTEINS

Colostrum obtained within a few hours after parturition was found to have a protein concentration between 15 and 26 per cent or, roughly, two to three times the concentration of blood plasma (25). By electrophoretic analysis, the immune protein may represent as much as 50 to 60 per cent of the total protein in colostrum, and as high as 85 to 90 per cent of the protein in colostrum whey. Therefore, it was a comparatively simple matter to isolate the immune protein in electrophoretically homogeneous form. After removal of the casein (Fraction *A*) by isoelectric precipitation at pH 4.5, the filtrate was brought to pH 6.0 with 0.5 M sodium hydroxide, and successive fractions were removed at 0.3 (Fraction *B*), 0.5 (Fraction *C*), and 0.9 (Fraction *D*) saturation with ammonium sulfate. After reprecipitation of each fraction within the same limits of salt concentration, the preparations were dialyzed and dried from the frozen state. Figure 1 shows the electrophoretic patterns obtained with the four fractions and with the original colostrum.

The electrophoretic pattern of the whole colostrum shows only a few components, with the slow-moving large peak due to the immune protein. The crude casein of colostrum (Fraction *A*) is complex in nature, like that of milk (15, 35), and contains at least two components (25). Fraction *B* consists entirely of a slow-moving globulin, and Fraction *C* of about 85 per cent of this protein. All of the immune activity of the colostrum was found to be associated with the lactoglobulin of low electrophoretic mobility (-1.8 to -2.2×10^{-5} sq. cm. per volt per second at pH 8.4).

The lactalbumin (Fraction *D*) is complex in nature and, like the similar fraction of milk whey, consists mainly of the β -lactoglobulin isolated by Palmer (18). The crystalline β -lactoglobulin of colostrum, as far as could be determined, is identical with that obtained from milk (25).

Quantitative isolation of the immune lactoglobulins was accomplished by precipitation at 0.4 saturation with ammonium sulfate after isoelectric precipitation of the casein. After this fraction was reprecipitated several

times under the same conditions, the material was homogeneous. Prolonged dialysis of the protein resulted in a separation of water-insoluble and water-soluble portions or eu- and pseudoglobulin fractions.

Figure 2 shows the electrophoretic patterns obtained with the normal whey of the later milk. Here the immune globulin represents only about 10 per cent of the whey protein in the normal animal, although the pro-

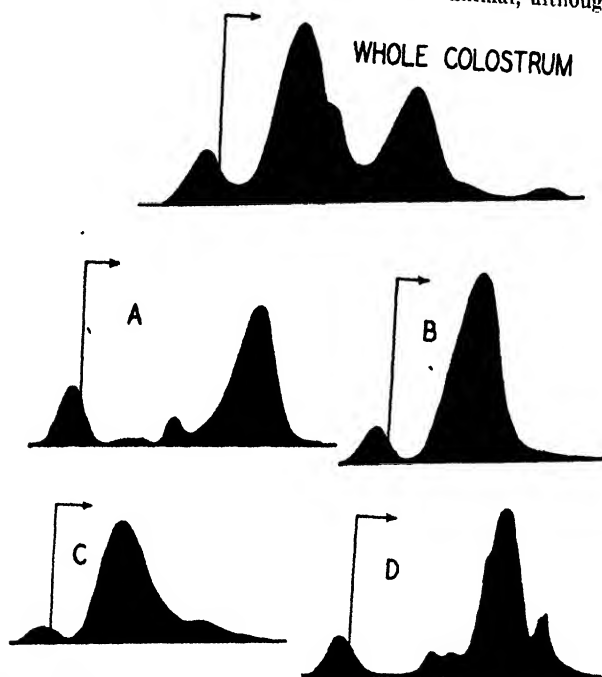


FIG. 1. Electrophoretic patterns of the descending boundaries of whole colostrum and of fractions derived from it. *A* is the casein; *B*, *C* and *D* are ammonium sulfate fractions obtained between 0 and 0.3 saturation (*B*), between 0.3 and 0.5 (*C*), and between 0.5 and 0.9 (*D*), respectively. Fraction *B* consists entirely of immune globulin and *C* mainly of this protein. The principal component of *D* is β -lactoglobulin, which could be obtained in crystalline form. Electrophoresis was for 200 minutes in veronal buffer at pH 8.3 to 8.4 at an ionic strength of 0.1. (Figure reproduced by permission of the Journal of Biological Chemistry.)

portion may increase somewhat in animals that have been hyperimmunized (26). The changes in protein composition during the transition from colostrum to milk have been studied by Crowther and Raistrick (5). More recently these changes also have been observed in the Tiselius apparatus (7, 14).

Because of the low concentration of immune protein in the milk whey,

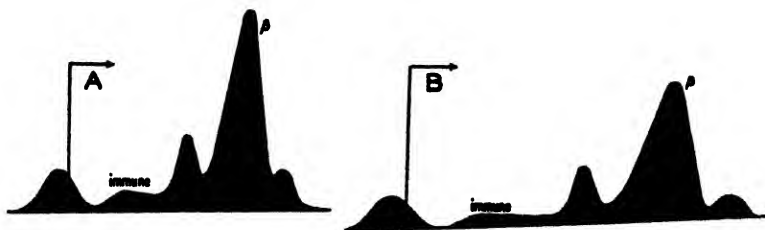


FIG. 2. Electrophoretic patterns of normal whey taken at 166 minutes (*A*) and 250 minutes (*B*). The principal component is β -lactoglobulin. The immune globulin represents about 10 per cent of the total protein. (Figure reproduced by permission of the Journal of Biological Chemistry.)

a somewhat different procedure from that used for the colostrum was adopted in order to isolate the immune lactoglobulin. This was accomplished

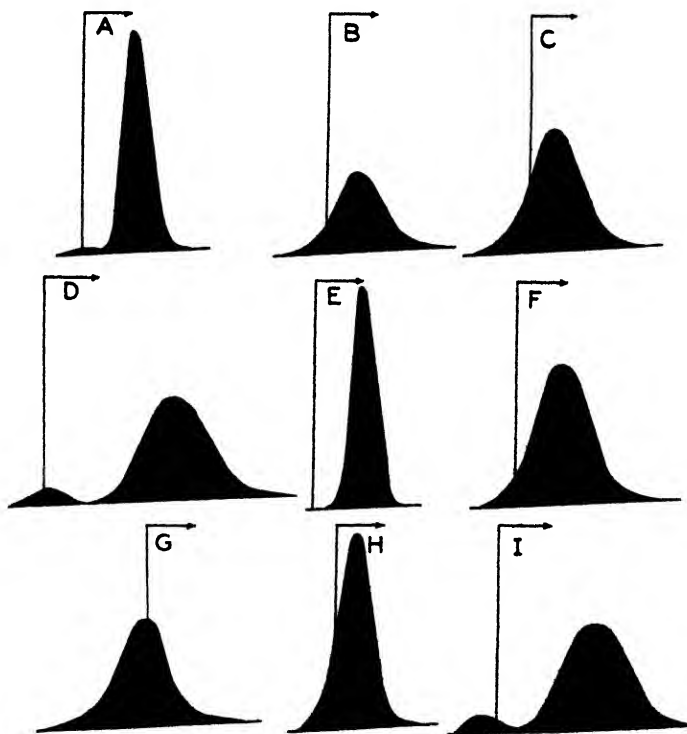


FIG. 3. Descending patterns of the purified immune lactoglobulins. The pseudo-globulin is shown in *A* (pH 3.90), *B* (pH 5.11), *C* (pH 6.81), and *D* (pH 8.55). The euglobulin is in *E* (pH 3.81), *F* (pH 5.12), *G* (pH 6.13), *H* (pH 6.82), and *I* (pH 8.65). These boundaries do not show the presence of the other milk proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

by ammonium sulfate fractionation involving isoelectric precipitations at different pH values (26). The immune lactoglobulins of milk and colostrum, as far as the authors have been able to determine, are extremely similar or, more probably, identical. Figure 3 shows the electrophoretic patterns obtained at various pH values with some of the purified immune lactoglobulins. These proteins are free from the other milk proteins. How-

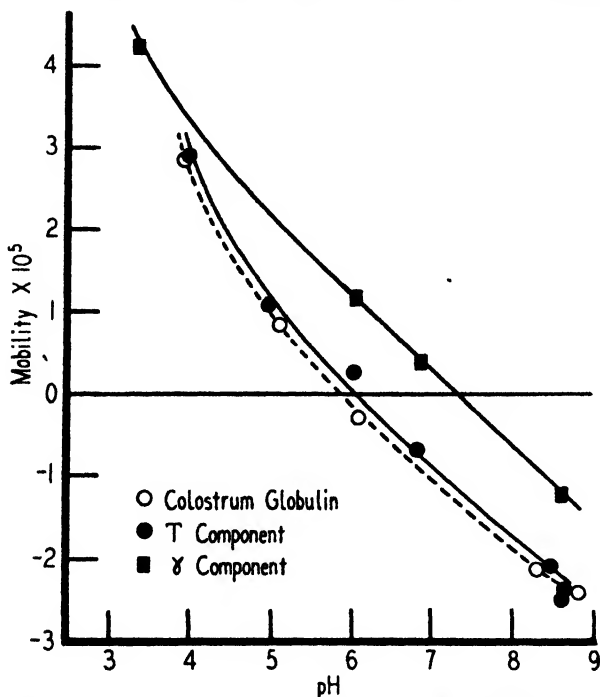


FIG. 4. Electrophoretic mobility as a function of pH for colostrum globulin, T-globulin, and γ -globulin. All of the values were calculated from descending migrations in univalent buffers at 1° C. The mobility is in sq. cm. per volt per second. The isoelectric point of the γ -globulin is at pH 7.2, that of the T-component at pH 6.15, and that of the total immune globulin of colostrum at pH 5.85. The separated lactoglobulins (not shown in the figure) gave for the pseudoglobulin an isoelectric point of pH 5.6, and for the euglobulin an I.E.P. of pH 6.05. (Figure reproduced by permission of the Journal of Biological Chemistry.)

ever, most of the boundaries show greater spreading than would be expected for a single molecular species. This is similar to the many observations that have been made with the γ -globulins of serum.

The animals from which samples of milk and colostrum were obtained had been hyperimmunized. All of the immune activity is associated with the specific lactoglobulins which were isolated and not with any of the other proteins of milk or colostrum, such as casein or β -lactoglobulin.

Since the immune proteins of bovine serum had not been previously isolated, it was necessary to develop a procedure for this purpose. As in the plasma of the horse (28, 34), immune activity is associated with the more rapidly migrating *T*-fraction as well as with the γ -globulin (25). The procedures developed by Cohn *et al.* (4) and Oncley *et al.* (16) for the fractionation of normal human plasma were adapted for the fractionation of hyperimmune bovine serum. This permitted the isolation of the γ - and *T*-globulins in electrophoretically homogeneous form (25). With these proteins in hand, it then was possible to compare the bovine immune proteins obtained from the different body fluids.

Figure 4 shows the electrophoretic mobilities as a function of pH for the bovine immune proteins. The γ -globulin possesses a much higher isoelectric point than the *T*-globulin or the total colostrum immune lactoglobulin. The eu- and pseudoglobulins of the colostrum or milk have slightly different isoelectric points, but these fall on either side of those of the *T*-globulin. From these facts alone it is possible to say that the immune proteins found in milk and colostrum are not γ -globulins as defined in terms of electrophoretic mobility and isoelectric point. However, it is not possible from these measurements to differentiate the lactoglobulins and the *T*-globulins.

While none of these proteins is completely homogeneous in the ultracentrifuge, they contain roughly 80–90 per cent of a component which sediments at 7 Svedberg units. The diffusion constants of these preparations range from about 3.3 to 3.9×10^{-7} sq. cm. per second. From these values it may be calculated that the principal components of the bovine immune lactoglobulins and serum globulins possess molecular weights in the neighborhood of 180,000. The ready diffusibility of the immune lactoglobulins of the colostrum through the intestine of the newborn animal cannot be due to any difference in size of these proteins as compared to the immune globulins of the serum.

Some studies have been made of the carbohydrate and amino acid content of the different bovine immune globulins (29, 30). All of these proteins were found to contain hexose and hexosamine in a ratio of about 2 to 1. These proteins, as shown in table 1, contain all of the amino acids known to be required in mammalian nutrition. In general, the data are similar to the analyses of human γ -globulin reported by Brand *et al.* (1). The immune globulins from horse and human serum also have been analyzed (29, 30); these greatly resemble the bovine proteins in their amino acid composition, although there are enough differences to indicate the different species from which the proteins are obtained.

It is rather striking that the immune proteins of different species appear to form a definite homologous group, with extremely similar physical and

chemical properties, in much the same way as do the serum albumins and the hemoglobins. It also is noteworthy that the immune globulins are quite different in amino acid content from any of the other proteins which are known to be present in mammalian milk or serum.

Although the bovine immune proteins greatly resemble one another, it is possible to distinguish between them. For example, attention may be called to the values for arginine and methionine of γ -globulin, which are much higher than those for the *T*-globulin or the lactoglobulins. The leucine content of the different proteins also shows marked differences. The phenylalanine content of the *T*-globulin is higher than that of the γ -globulin

TABLE 1

Amino acid and carbohydrate content of bovine immune proteins

(Data are averages derived from (25), (26), (29) and (30). All values are in terms of the anhydrous ash-free proteins.)

Constituent	Eu-lacto- globulin	Pseudo- lactoglobulin	<i>T</i> -globulin	γ -globulin
	(%)	(%)	(%)	(%)
Arginine	4.9	3.5	4.8	5.8
Histidine	1.89	2.14	2.01	2.05
Lysine	6.3	7.2	6.4	6.7
Isoleucine	3.1	3.1	3.0	3.2
Leucine	10.4	9.1	8.6	7.4
Valine	10.4	9.4	9.5	10.0
Threonine	10.5	10.1	9.5	10.0
Phenylalanine	3.6	3.8	4.5	3.2
Tryptophane	2.4	2.7	2.6	2.6
Cystine	3.2	3.0	2.8	2.9
Methionine	0.98	1.08	1.00	1.18
Sulfur	1.05	1.04	0.95	1.02
Hexose	2.9	2.8	2.5	2.05
Hexosamine	1.45	1.32	1.50	1.31

or of the immune lactoglobulins; this is strikingly reflected in the ultraviolet absorption spectra of these proteins (27). As shown in figure 5, bovine *T*-globulin possesses a much steeper end-absorption than the colostrum or γ -globulin. It thus appears that the lactoglobulins possess somewhat different protein moieties than the serum immune proteins.

However, the lactoglobulins and the serum globulins must be substances very closely related. All of these proteins will give quantitatively equivalent anaphylactic cross-reactions in guinea pigs sensitized to bovine immune proteins whether they are derived from milk or from serum (25). It is well known, particularly from the works of Wells and Osborne (36), that the globulin fractions of milk and serum are immunologically related. It now has been shown that these cross-reactions are due to the immune proteins.

The presence in the immune proteins of all the amino acids known to be required for the maintenance of nitrogen equilibrium in mammals raises

an important nutritional question. Obviously, for protection of the adult and the newborn against infectious disease, all of the so-called essential amino acids must be supplied in adequate amount. Evidence is available which shows that hypoproteinemia causes a decrease of antibodies and a lower resistance to infection. The synthesis of the globulins concerned with immunity is then a special problem in nutrition, as recently emphasized by Cannon (3).

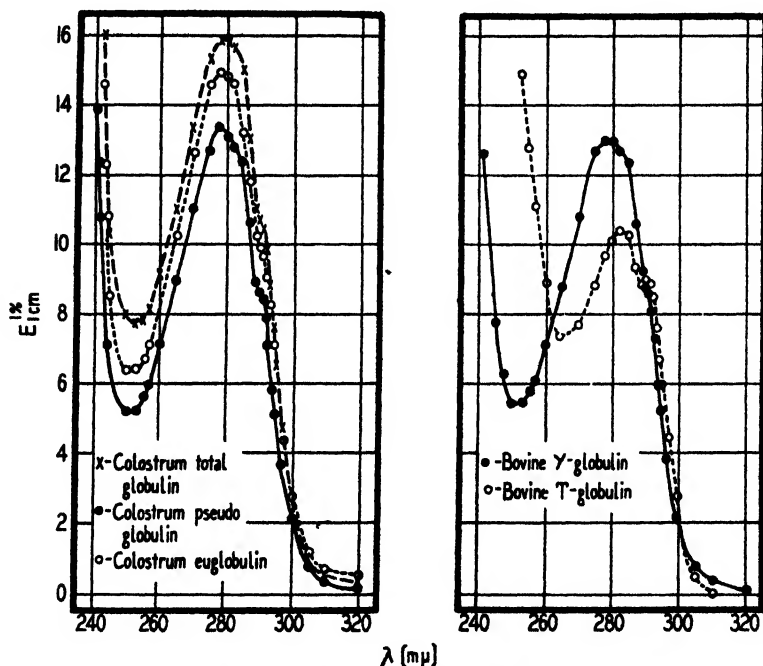


FIG. 5. Ultraviolet absorption spectra of bovine globulins. The absorption curve of the T-globulin appears to reflect the higher content of phenylalanine by the steeper end-absorption as compared to the other proteins. (Figure reproduced by permission of the Journal of Biological Chemistry.)

PASSIVE IMMUNITY IN THE CALF

Since it already has been demonstrated that the colostrum immune globulin is different from γ -globulin in electrophoretic mobility and other properties, it is preferable not to refer to the globulin which appears in the blood stream of the newborn after the ingestion of colostrum as a γ -globulin. The protein which appears in the blood stream of the calf after ingestion of colostrum possesses the electrophoretic mobility of the immune lactoglobulin and not that of γ -globulin (31). Figure 6 shows the electrophoretic patterns obtained with the serum of the newborn calf at birth and

at various times later. The calf was fed colostrum only during the first day of life, and thereafter was isolated from the mother. The serum of the newborn calf did not contain any slow-moving globulin. However, the pattern of the serum obtained 2 days after birth showed a large amount of colostrum globulin (44 per cent of the total protein). Thereafter, the

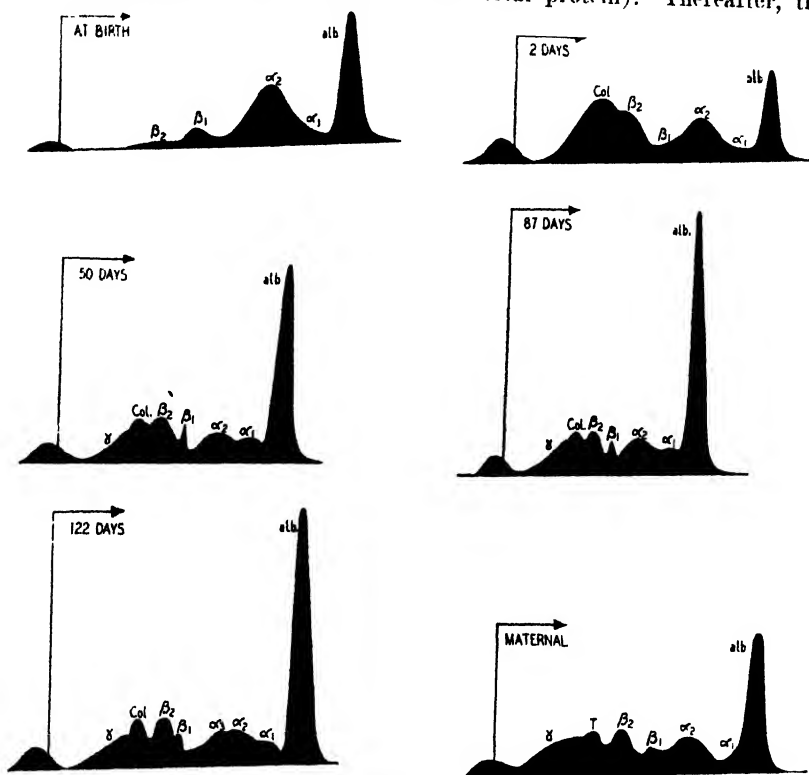


FIG. 6. Electrophoretic patterns of the descending boundaries of the serum of a newborn calf and of the same animal 2, 50, 87 and 122 days later. For comparison, the maternal serum obtained 3 days before term also is shown. The runs were performed at 1° C. in a veronal buffer of pH 8.4 to 8.6, and at an ionic strength of 0.1. The serum of the newborn is practically devoid of slow-moving globulins. The immune component (Col.) appears after feeding colostrum. The absolute heights of the different serum samples cannot be compared as the runs were made at somewhat different protein concentrations.

amount of colostrum globulin decreased steadily. From such data it is possible to estimate the time during which this protein remained in the blood stream of the calf. The immune component decreased to about one-half its initial concentration in about 20 days, and persisted for many

months. γ -globulin was hardly detectable in the blood stream of the newborn calf. Even after 4 months, it had not reached the normal adult level. It is obvious that the passively acquired immunity is of real importance to the health of the calf for the long period before it is capable of making antibodies of its own.

It also should be stated that the mobilities and relative concentrations of the various serum proteins in the newborn calf may be very different from those in the adult. This should be extremely useful in approaching some of the problems of the physiology and chemistry of the fetus. In fact, Pedersen (20) recently has reported the isolation of fetuin, an α -globulin, from the serum of the bovine fetus. Bovine fetal hemoglobin reportedly differs from that of the adult (37).

SUMMARY

Colostrum serves a special function in order to enhance the resistance of the newborn to infectious disease. This is shown by the extremely high concentrations of immune lactoglobulins in the colostrum. These globulins are passively transferred to the offspring, where they may persist in the blood stream for many months. The lactoglobulins which have molecular weights near 180,000 pass from the intestinal tract of the calf to its blood stream. The immune lactoglobulins of bovine milk and colostrum, and the γ - and T -globulins of bovine serum, have been isolated and compared with respect to their physical and chemical properties.

CONCLUSIONS

It is clear that the colostrum serves a special function in order to enhance the survival of the newborn animal. Not only is the colostrum richer than the milk in some of the vitamins, as demonstrated by various investigators (6, 19, 23), but it also contains a totally different distribution of proteins than the milk. The extremely high percentage of immune lactoglobulins, together with the fact that the colostrum may contain 25 per cent protein in the aqueous phase, demonstrates the extreme nature of this adaptation. It is not surprising that many investigators have found that colostrum generally possesses higher immune titers than the maternal blood (2). The long duration of the passively acquired immunity in the calf also emphasizes the great importance of colostrum to the health and well-being of the newborn.

The protein synthesis of the mammary gland presents an intriguing picture. The gland makes special proteins such as casein and β -lactoglobulin, which are not found elsewhere in the body, and even gives its own characteristic label to the immune bodies which must be drawn from the blood stream.

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THE UTILIZATION OF WHEY: A REVIEW

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The purpose of this review is to make available in one article pertinent information on whey and its constituents and on procedures for the manu-

facture of both food and non-food products from whey. Sufficient details are given so that cheese producers can select processes suited to their individual locations and scales of operation.

THE WHEY DISPOSAL PROBLEM

More than ever before producers of cheese are seeking new methods of utilizing whey. The reasons for this are apparent. Older methods of disposal are becoming less available and several other possible methods are prohibitively expensive to operate. The following procedures either have been employed or have been advocated for disposal of dairy wastes (124):

(a) Running to the sewer. This is practical only when the quantity of whey is very small or the whey can be diluted greatly; otherwise, a nuisance is created or, if the sewage is treated, an inordinate load is placed on the treating plant. Since the biological oxygen demand of whey is high and the quantity usually is large, this method is not feasible in most instances.

(b) Running to a stream. Any appreciable quantity of whey will pollute a small stream sufficiently to kill fish and to produce noxious odors in stagnant areas.

(c) Dumping in abandoned mines or quarries or in holes dug for disposal purposes. The cost of transporting whey to abandoned mines or quarries will be prohibitive in most instances and the odors produced by the decomposing whey will be objectionable unless the place of disposal is at a considerable distance from the cheese plant and from homes.

(d) Dumping in prepared lagoons. Practically the same objections apply to lagooning or spreading on fields as to the preceding method.

(e) Treating in a sewage disposal plant installed for the purpose. The cost of a disposal plant for whey will be unreasonably great because of the high value of the biological oxygen demand of the whey.

(f) Producing fuel gas by anaerobic fermentation. It appears theoretically possible to produce all the heat required in a cheese plant by this procedure, but such a heat source probably would be unreliable. Digestion tanks of about 30 times the daily volume of whey would be required.

(g) Returning the whey to farmers for feeding. This is practical, but only to a limited extent. The large volume of the whey in relation to the quantity of nutrients present and the requirement that whey may not be transported to the farms in the cans used for bringing milk to the plants discourage many farmers from using whey for feeding. Furthermore, the farmers having pigs to feed usually are not the same ones as those delivering milk to the cheese plant.

(h) Evaporating or drying for use as food or feed. Where cheap heat is available and the volume of whey is great enough to justify investment in evaporating or drying equipment, this is a practical means of disposal.

(i) Manufacturing of such products as whey protein, whey cheese, lac-

tose, lactic acid, alcohol, vinegar and food specialties. This and the preceding method are the most desirable from the standpoint of nutritional economy and usually can be operated at the least net cost, and frequently at a profit. The special equipment required is expensive.

QUANTITIES OF WHEY PRODUCED

Approximately 10 billion lb. of whey are produced each year in the United States. About 9 billion lb. are from the manufacture of whole milk cheese and one billion lb. from cottage, pot and bakers' cheese. The 40 million-lb. quantity of whey from the making of casein, though relatively small in amount, is of importance because, until recently, it has been the source of all the lactose produced in this country.

Ten billion pounds of whey contain, in round numbers, 500 million lb. of lactose, 50 million lb. of protein, 40 million lb. of non-protein nitrogenous matter, 30 million lb. of fat, 11 million lb. of phosphorus (P_2O_5), 7 million lb. of calcium (CaO), and 12 thousand lb. of riboflavin. Forty million pounds of ash constituents also are present but are of little or no interest from the standpoint of utilization. The fat can be recovered readily by means of a cream separator for use in making butter and therefore does not contribute to the whey disposal problem.

COMPOSITION AND NUTRITIVE VALUE OF WHEY

A typical cheese whey contains 6.9 per cent total solids, of which percentage 0.6 is ash and 6.3 is organic solids, divided among 0.3 per cent fat, 0.9 per cent nitrogenous compounds (calculated as protein), 4.9 per cent lactose and 0.2 per cent lactic acid. The lactic acid has been formed by fermentation of lactose, and the percentages of these two constituents are somewhat variable, but their sum is consistently close to 5.1 per cent. About five-ninths of the nitrogenous matter is heat-coagulable protein. This protein commonly is called either whey protein or albumin, but the term albumin is an improper one since this material consists of a very small proportion of suspended casein, an "albumin fraction" and a "globulin fraction". These fractions differ in both composition and physical properties and can be fractionated still further.

For the purposes of this review, the term whey protein will include all of the heat-coagulable protein of whey, and its heterogenous composition will be disregarded. The nitrogenous matter that is not coagulable by heat consists of substances precipitable by trichloroacetic acid, which are peptone or proteose in nature, and other simpler substances such as creatin, creatinin, urea, uric acid, amino acids and ammonia. Of the known vitamins, the only one present in appreciable quantities in whey is riboflavin, which occurs to the extent of approximately 1.24 γ per g. of whey, or 0.000124 per cent (25).

The chief individual ash constituents present in whey are: 0.188 per cent

potassium oxide, 0.075 per cent sodium oxide, 0.071 per cent calcium oxide, 0.018 per cent magnesium oxide, 0.001 per cent ferric oxide, 0.110 per cent phosphorus pentoxide, 0.107 per cent chlorine and 0.029 per cent sulfur trioxide. Of these, the calcium and phosphorus are of positive interest because of their nutritional value. The other salt constituents usually have only nuisance value because of the salty flavor that they impart to concentrated whey products and the difficulty of removing them.

Milk is unique as a source of calcium and riboflavin, two nutrients that need to be increased in many American diets. It is unique also in that it contains lactose, a sugar having highly specific nutritive value. These three nutrients largely are left in the whey from the cheesemaking process, together with part of the phosphorus. The whey protein is of excellent quality in that it contains practically all of the essential amino acids.

Lactose brings about increased utilization of calcium, magnesium and phosphorus in young animals (35, 72, 81). This effect may be the real basis for many claims as to the superior assimilability of calcium and phosphorus from whey products. Lactose, unless fed in excessive quantities, is more effective in accelerating growth in young animals than are other common carbohydrates (141). It favors the production of riboflavin and vitamin B₆ in the intestine of the rat (74). The feeding of lactose to rats has caused cataracts, but it has been found that fat, which is necessary for the utilization of dietary lactose, protects against development of cataracts (51, 103). Young rats die when fed lactose in high concentration as the only carbohydrate in the diet (31, 40) unless an unidentified factor associated with casein is present (18). It should be understood that these undesirable effects of lactose have been obtained only in rats and then only on diets that were highly abnormal.

In the poultry industry, dried whey is fed extensively because lactose is effective in preventing coccidiosis, and riboflavin is considered essential to the rapid growth of chicks and to hatchability of eggs and is a preventive of curled toe paralysis.

PROCESSES FOR PRIMARY PRODUCTS

Methods of whey utilization discussed in this review are shown in figure 1. Three primary processes are employed in the preparation of whey for ultimate utilization in feeds, foods, pharmaceuticals, or industrial products. The different primary and final products will be considered here approximately in the order in which they appear in the figure.

If whey is to be processed, the initial processing should be carried out in the plant in which the whey is produced in order that deterioration due to the growth of undesirable organisms will be retarded or prevented. Such treatments may include one or more of the unit operations of pasteurization, concentration or fermentation.

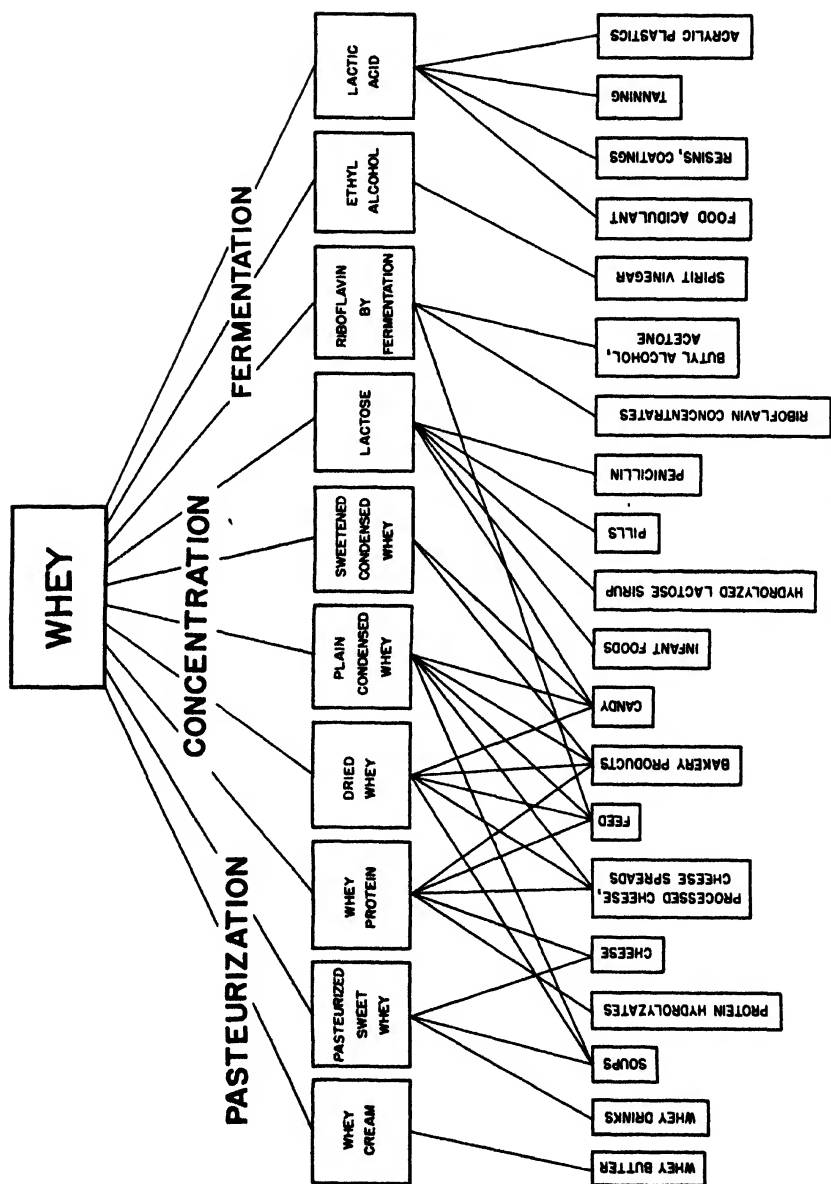


Fig. 1. Flow sheet of products from whey.

Pasteurized Products

Sweet whey. Pasteurization is an essential step in the preservation of sweet whey for further processing and in the production of whey cream. Whey is heated to 145° F. for 30 minutes, or to higher temperatures for shorter times, to retard growth of lactic-acid-producing bacteria which have been active in the cheesemaking process and to destroy pathogenic organisms that may be present if the milk was not pasteurized prior to making the cheese. The whey should be cooled to below 50° F. immediately after pasteurization, unless additional processing is to ensue promptly.

Pasteurized sweet whey is bulky and perishable, but, where there is a convenient source of supply, it can be used successfully in foods. It may be used to advantage in place of water in recipes for beverages, soups, bread and other foods.

Whey cream. It is prepared by putting whey through a cream separator either before or after pasteurization. Products of bacterial growth that produce off flavors may be present in whey that is not properly pasteurized and may be transferred to the cream. When cheese is made in copper equipment, cream made from the whey often has a copper content great enough to catalyze the oxidation of the fat; as a result, off flavors appear in butter or other products made from the cream.

The danger of curdling during the pasteurization of mixtures of whey cream and cream from whole milk can be lessened or avoided by using whey cream of low acidity or by pasteurizing the whey cream separately before it is mixed with the cream from whole milk (123).

Although butter most generally is the product made from whey cream, the cream may be used in foods in which casein coagulates readily. Whey cream contains substantially no casein and will not produce a coagulum when added to cold, acid foods. The coagulum formed during heating is soft and easily dispersed.

Concentrated Products

To remove water from whey, two methods are in use, one employing vacuum evaporators and the other driers. Both require from 40,000 to 100,000 lb. of whey daily for profitable operation. However, if equipment already is available and if the whey cannot be discarded, it may be practicable to concentrate as little as 10,000 lb. of whey daily. The engineering and operating aspects of concentrating equipment have been considered at length by Hunziker (46), Scott (104) and Farrall (32).

Plain condensed whey. This is the simplest of the whey concentrates to manufacture (136). Pasteurized whey is condensed in a vacuum pan to about 68 per cent solids (36.7° Baumé at 115° F.), dropped into cans, barrels or a tank, seeded with lactose or a concentrate from a previous run, and, if possible, cooled with agitation. A multiple-effect evaporator will

condense whey to 40 or 50 per cent solids; a single-effect pan can reduce it to 70 per cent solids. When whey is being condensed to 70 per cent solids, there is a possibility that the concentrated solution suddenly will crystallize in the pan and that the heating surfaces will become badly coated with whey solids (60). To avoid this difficulty, the pan should be clean and free of lactose crystals at the start of each run and the batch should be quickly finished and dropped from the pan (136).

Excessive foaming of whey in the vacuum pan sometimes occurs. The addition of a small quantity of milk fat or other fat to the batch usually will reduce this foaming. Triggs (125) found that if the whey was adjusted to pH 5.5 to 6.0 before it was drawn into the vacuum pan, foaming could be practically eliminated. The acid preferred was phosphoric, and this was neutralized with lime as soon as the concentrated whey was dropped from the pan.

Sweetened condensed whey. This was developed to provide a simple and economical method for the preservation of whey solids for use in food manufacture (92, 137). A quantity of sugar equal in weight to that of the solids in the batch of whey is added to the whey after it has been run through the separator and pasteurized. The mixture is condensed under vacuum to 76 per cent solids (38.4° Baumé at 122° F.). The concentrate is cooled to 95° F., seeded with lactose crystals or with a concentrate from a previous run, stirred for 1 hour and placed in barrels or cans. If a product containing a reduced content of lactose is desired, crystallized lactose may be removed centrifugally before the concentrate is stored. The storage requirements, as well as the manufacturing procedure, are similar to those for sweetened condensed milk. The whey product thickens more slowly and to a lesser extent than does the milk product. Fat-free sweetened condensed whey may be whipped to a dense foam having 200 per cent overrun and a stability of several hours.

Dried whey. When produced by the processes employed in drying milk, dried whey cakes on standing because the anhydrous lactose present gradually absorbs water and crystallizes as a hydrate (108, 110). Many patented processes have been devised principally for the purpose of inducing lactose crystallization prior to the complete drying of the whey. Some of these processes may be applied in either the roller or spray drying procedures.

Processes for controlling the crystallization of lactose during the drying of whey may be divided into four groups: (a) Whey is concentrated to a solids content of 70 per cent or more, the lactose is allowed to crystallize, and then the mass is dried (11, 53, 60, 111). (b) The whey is dried and then allowed to absorb water to force the crystallization of the lactose (20, 30, 87, 130). (c) The whey is concentrated to the point at which it

contains approximately the quantity of water required for hydration of the lactose (6, 58, 59, 85, 88). No water is added and under carefully controlled conditions it is not necessary to remove water after the crystals have formed. In practice, however, often about 2 to 5 per cent of water must be removed after crystallization of lactose is complete. (d) A dried whey in which lactose is largely in the *beta* anhydride form is prepared by seeding a partially concentrated whey with *beta* lactose at a temperature above 200° F. and holding it at this temperature while it crystallizes and dries (20, 59).

Three general drying methods are employed in the manufacture of dried whey: tunnel or shelf drying, drum or roller drying, and spray drying. Since many of the processes and their modifications employed in the drying of whey are patented, the status of the patents in this field should be investigated before manufacturing operations are started. Bosworth (12) concentrated whey under vacuum and then dried it in air at 149° F. for food and pharmaceutical uses.

The commercial method of concentration and tunnel drying of whey is based on the Simmons patent (111), which only recently has expired. Whey is condensed under vacuum to 70 per cent solids, dropped from the vacuum pan, seeded and held not more than 24 hours to allow the lactose to crystallize. The pasty mass then is dried in a tunnel drier and ground. The finished product is relatively non-hygroscopic and the whey protein is insoluble in water. In the Kraft modification of the Simmons process (53), the whey concentrate containing 70 per cent solids is dried in air by blowing it through a series of conduits and cyclone collectors. The lactose crystallizes during this drying operation. Lavett (60) concentrates whey of 40 per cent solids to 80 per cent on a double drum drier, drops this to cooling and seeding drums, and then to a hot-air drier. Bertram and Lemmerich (11) pass the concentrated whey through a mixing machine in which it is mixed with air and the lactose crystallized. The material then is dried and ground. Peebles and Manning (89) claim that, by heat-coagulating the whey proteins prior to crystallizing the lactose in the concentrate, the protein then will not interfere with crystal formation, and a stable, non-hygroscopic dried whey will be produced.

Drum or roller drying of whey can be carried out by following conventional methods (3), but modifications generally are employed to retard the formation of a sticky, hygroscopic glass on the drums. Lavett (57, 59, 60) uses two double-drum drying units placed one above the other. Whey is concentrated in a vacuum pan to 40 per cent solids and then reduced on the upper drums to 80 per cent solids. The mass is cooled and seeded on the lower drums and finally dried in a rotary drier. When a single pair of rolls is employed, adjustment of the titratable acidity of the whey to be

tween 0.30 and 0.40 per cent before drying is helpful (61). In one modification, the drying mass is stripped from the drums when it contains from 8 to 15 per cent moisture, the lactose allowed to crystallize, and the drying finished by means of hot air (58).

Drying aids may be added to the whey. Pectic acid is used in the drying of mixtures of whey and fruit juices (143). The dried whey can be scraped off the rollers easier if a finely divided dry material, such as dried whey, is sprinkled on the semi-dried film as the rolls revolve (6). Spellacy (115), Supplee (119), and Jack and Wasson (48) found that whey dried with less difficulty when it was mixed with a material which formed a sheet as it was scraped from the drums. Skim milk, buttermilk and organic, water-insoluble, non-gelatinized substances such as ground and sifted cereals were found to be suitable drying aids. Waite (128) found that cheese whey and hydrochloric acid-casein whey could be drum-dried if they were neutralized with calcium hydroxide, but acetic acid-casein whey could be dried without neutralization.

Spray drying can be accomplished by the processes used for drying milk. However, the hygroscopic nature of spray-dried whey sometimes causes it to cake in the collection system of the drier and obstruct the passages. This tendency is lessened by inducing lactose crystallization during drying by an adaptation of one of the procedures previously described.

The addition of pectic acid to whey (143) and heat-coagulation of the whey protein (89) have been recommended as preliminary treatments for whey that is to be spray-dried.

Casein sometimes is removed from skim milk by treatment with one of several gums (2). The resulting whey is highly viscous; it may be treated with an enzyme that will act on the gum, thus reducing the viscosity of the whey and making it easier to handle in drying equipment (23).

Dried whey often gradually becomes brown subsequent to drying. Doob *et al.* (28) found this objectionable change to be associated with a high content of osmotically held moisture, high titratable acidity and low lactose content.

Lactose. Until recently, lactose was made in this country only from muriatic casein whey. However, the increased demands for lactose in the manufacture of penicillin during World War II, together with the simultaneous decrease in the quantity of casein whey available, made it necessary to use cheese whey as a source of lactose. The difficulties in processing cheese whey for lactose manufacture had been overcome experimentally, and several procedures were available for commercial use. The methods employing casein whey and cheese whey are outlined here.

Whey from casein precipitated by means of sulfuric acid is objectionable

because of the difficulty of removing slightly soluble metal sulfates that impart cloudiness to lactose solutions. Self-sour casein whey is not recommended because so much of the lactose has been converted to lactic acid that yields will be low. The same is true to a less degree of cottage cheese whey. In general, the less the fermentation that has taken place in the whey the greater the yield of lactose that will be obtained.

The whey protein is recovered in an insoluble condition in the process using casein whey and in those processes using cheese whey wherein the whey is clarified by boiling. This insoluble protein is suitable for feed. When a soluble protein suitable for use as food is desired, the whey should not be boiled; under these conditions the lactose obtained will contain a relatively high percentage of protein and ash, usually 1.5 per cent or more of each. Two stages of clarification and boiling are necessary to produce, with one crystallization, lactose containing less than 0.3 per cent protein and 0.3 per cent ash. The recovery of lactose usually is 3.5 to 4 lb. per 100 lb. of whey. A second crystallization is necessary in order to produce USP lactose. For many purposes, however, lactose of crude or technical grade is satisfactory and is less costly to produce.

Muriatic casein whey (78, 117) is heated to boiling in iron tanks with live steam, and lime is added during the heating until the acidity is about 0.5 per cent or the pH value is 6.2. The coagulum is allowed to settle and the clear whey is evaporated in a double-effect evaporator to a concentration of 30 per cent lactose, or 20° Baumé. The hot sirup is filtered in a filter press and is followed by the sludge from the coagulating tank. The clear sirup then is evaporated in a single-effect evaporator to about 40° Baumé, some crystallization or "graining" taking place in the evaporating pan. The hot mass is dropped to crystallizing vats, where it is agitated slowly and cooled by water circulating in a jacket. The sugar is freed from mother liquor by spinning in a sugar centrifuge and then washed with cold water. A second crop of crystals can be obtained by concentrating the mother liquor. The wet crude lactose either should be refined or dried promptly to avoid spoilage.

The simplest method of making lactose from cheese whey (9, 10, 138) is to concentrate it in a vacuum evaporator to 55-60 per cent solids content, cool the concentrate with occasional stirring in a vat, separate the lactose in a centrifuge, wash with cold water, and dry in a tunnel drier. The resulting crude sugar will contain approximately 5 per cent impurities (protein and ash) on a dry basis. The whey protein in the mother liquor will be soluble.

Better grades of lactose are obtained when the heat-coagulable fraction is removed from the whey before concentration. During evaporation of the clarified whey further precipitation of insoluble protein and salts occurs.

If the sirup is filtered when its concentration reaches 20° Baumé, or about 30 per cent solids, there will be a further improvement in the finished product. The 20° Baumé sirup also may be decolorized with activated carbon and bone-black to produce a colorless lactose. The resulting technical grade sugar will contain more than 99 per cent lactose and only 0.3 per cent each of protein and ash on the dry basis. A lactose that will not foam in solution may be made by digesting the clarified whey with the enzyme trypsin before concentration.

Several other methods that have not come into commercial use have been described in the literature (142).

For refining (118), crude sugar is dissolved with the aid of steam in sufficient water to give a concentration of 20° Baumé. One pound of decolorizing paste and 0.25 lb. of a filter aid are added for each 100 lb. of sugar present. The solution is heated to boiling and hydrochloric acid is added to give a titratable acidity of 0.09 per cent, expressed as lactic acid. After standing overnight, the batch is heated nearly to boiling and milk of lime added to reduce the acidity to 0.05 per cent, or a pH value of 5.4 to 5.8. The solution then is boiled vigorously for a few minutes and allowed to stand until the insoluble matter has settled. It then is filtered through cloth in a press and again through rag paper supported between perforated copper discs. The acidity of the filtrate is increased slightly by addition of hydrochloric acid, the solution is concentrated to 40° Baumé, and the sugar is crystallized, centrifuged, washed and dried. The product should satisfy the specifications for USP lactose.

Drying lactose solutions by the spray-drying process (8) produces a mixture of the two forms in approximately the equilibrium ratio of 1.65 parts *beta* to 1.00 part *alpha*. The product dissolves much more rapidly than *alpha* lactose but is hygroscopic and has poor wetting properties. The product made by drying lactose solutions on a drum drier will contain as much as 90 per cent of the sugar in the *beta* form under the most favorable drying conditions. Such a product is less hygroscopic than the spray-dried product, has good wetting properties, and is slightly more soluble initially than pure *beta* lactose.

In the process of Supplee and Flanigan (120), a solution of lactose is dried in a thin film at a temperature above the boiling point of water, the film is removed from the source of heat while it contains at least 2 per cent of water, and the heat remaining in the paste completes the drying. The product contains a high proportion of *beta* lactose.

In Sharp's process (106, 107), *alpha* lactose is added to a saturated lactose solution maintained above the critical temperature of 200.4° F. The *alpha* lactose dissolves and reappears as crystalline *beta* lactose, which is separated by filtration in a heated centrifuge.

In the Sharp and Hand process (109), dry *alpha* lactose is heated in a closed container at 248 to 266° F. Under these conditions, *alpha* lactose loses water of crystallization and changes to the *beta* form. When the conversion has reached completion, or a desired stage short of completion, the water vapor in the container is allowed to escape.

Whey protein. This easily can be recovered as a denatured protein from either concentrated or unconcentrated whey by heat coagulation, but the soluble product is difficult to isolate. Soluble whey protein generally is prepared from a whey concentrate from which the crystallized lactose has been removed. The remaining liquor contains the whey protein, some lactose, and the soluble whey salts, which are difficult to separate from the protein without causing denaturation. Patents were obtained by Dunham in 1902 (29) for precipitating whey albumin from concentrated whey by means of acid, by Weimar in 1921 (139) for preparing a soluble whey protein concentrate from which lactose was partly separated by crystallization and salts by dialysis, and by Meyer in 1931 (71) for removing salts by chemical means.

Bell and Peter (9) and Bell *et al.* (10) improved the Weimar process. Cheese whey is neutralized with sodium hydroxide to pH 7.3, condensed to 62 per cent solids, cooled, and centrifuged to separate the lactose. The mother liquor, which contains soluble whey protein, milk salts and residual lactose, is suitable for use in food preparations. Watson (131) was able to remove most of the salts from the mother liquor of the Bell, Peter and Johnson process by electro-dialysis. Perhaps this can be accomplished more readily by application of the more recently developed ion-exchange procedures, first advocated by Lyman (68).

Leviton (62) and Leviton and Leighton (67) extract soluble protein from whey by means of alcohol. Dried whey containing non-crystalline lactose rapidly is mixed with ethanol, and the protein, being insoluble in the alcohol, promptly is separated by filtration and dried. The dried product is soluble in water. Lactose crystallizes from the mother liquor and is recovered by filtration; the alcohol is recovered by distillation, leaving a residue relatively rich in riboflavin. Similarly, Leviton (66) has extracted protein from dried skim milk by means of methanol. The protein complex is redispersed in water and the casein is precipitated by acid or rennet, leaving soluble whey protein in solution.

Another method for the separation of water-soluble protein from whey was devised by Gordon (38). The protein is precipitated at pH 3 by addition of a soluble metaphosphate, separated by filtration, and washed and treated with excess calcium hydroxide at pH 9 to precipitate calcium phosphate. The mixture then is adjusted to pH 7 and centrifuged. The filtrate contains the whey protein and is evaporated under vacuum to yield the protein in a dry undenatured state.

Water-insoluble whey protein may be separated from whey by heat coagulation. Investigators have determined quantitatively the effect of reaction and temperature on the heat coagulation of protein in cheese whey (47, 76, 80). In general, 50 to 60 per cent of the nitrogen in whey is recovered as coagulated protein when the whey at a reaction between pH 4.5 and 5.0 is boiled. Several practical processes have been developed (15, 34, 130). According to Burkey and Walter (16), sweet whey (pH 6.3) is heated to 200° F. and acidified to pH 5.0 with any suitable acid or with sour whey. The sour whey used should contain 2 per cent lactic acid and is added in an amount equal to 10 per cent of the whey being treated. During acidification the whey is stirred; then it is held hot and without stirring for about 15 minutes. The clear liquid may be siphoned or drained off, or the flocculated protein dipped into cheese cloths and drained and washed. Curd that has been drained and washed contains more than 74 per cent moisture and may be preserved by drying or freezing. The clarified whey remaining after removal of the protein may be used for lactose manufacture or for animal feed.

Centrifugal separation and washing of heat-coagulated whey protein have been accomplished by means of specially built, high-speed centrifuges. The composition of the protein suspension produced may vary widely. One procedure produces a concentrate containing about 2 per cent whey protein and very small quantities of lactose and salts. Some of these suspensions resemble skim milk in appearance and can be used in food manufacture either directly or after concentration by vacuum evaporation or by drying.

Fermentation Products

The substances that can be produced by the fermentation of the lactose of whey can be produced by fermentation of cane, beet or corn sugar. Whether it is practical to utilize whey in making fermentation products depends in general on whether a suitable organism is available to convert lactose into the desired product and whether whey is a less costly source of fermentable sugar than is molasses or corn sugar.

Of the many substances that can be produced by fermentation of lactose, the only ones being produced in this country are lactic acid, ethyl alcohol and riboflavin. Lactose is used in penicillin production because its slow rate of acid production under the required conditions favors increased formation of penicillin, but it is not essential to the fermentation. Since lactose, rather than whey, is used in this fermentation, the process is not described here.

Lactic acid. It is produced commercially from whey by means of a mixed culture of a lactobacillus and a mycoderm, American Type Culture Collection no. 9223 (17, 49, 140, 142). The efficiency of conversion is greater than 90 per cent; the acid is the inactive mixture of the dextro and levo forms, and no objectionable by-products are formed.

A starter culture is built up by successive inoculations and incubations of batches of whey of increasing size. Five hundred gallons of starter are added to 5,000 gallons of raw whey maintained at 43° C. (110° F.). Every 6 hours, or whenever the reaction approaches pH 5.0, a slurry of slaked lime is added in quantity sufficient to bring the reaction to pH 6.0 but not higher. When chemical tests show that practically all the sugar has been fermented, or when the quantity of lime consumed indicates that the conversion is complete, the whey is neutralized to pH 6.5 to 7.5 with lime slurry and heated to the boiling point. After 10 minutes at the boiling point, the coagulum is allowed to settle, the clear liquid is run to a filter press and is followed by the sludge. The hot filtrate is treated with a small percentage of decolorizing carbon, stirred, and brought to a pH value of 10.0 by addition of lime slurry. As soon as a test on a sample shows that sedimentation will be rapid, the precipitate is allowed to settle and the batch again is filtered. The filtrate is neutralized with lactic acid and concentrated in a vacuum pan at 15° Baumé. The concentrate is run to jacketed crystallizers, and, by circulating cold water in the jacket, it is cooled to 10–15° C. (50–60° F.). After 12 hours, the crystalline mass is spun in a basket centrifuge until no more filtrate is obtained and the crystals are washed lightly with cold water. The mother liquor and washings are concentrated to 13.5° Baumé to obtain a second crop of crystals. The calcium lactate obtained may be recrystallized to produce USP calcium lactate or it may be treated with sulfuric acid to convert it to lactic acid either before or after crystallization.

Lactic acid comes on the market principally as 22 and 44 per cent crude, 50 per cent edible, and 65 per cent USP acid.

Ethyl alcohol. The production of ethyl alcohol from the lactose of whey (50, 77, 97) requires a lactose-fermenting yeast. *Torula cremoris*, American Type Culture Collection no. 2512, is the most efficient yeast found for the purpose; it produces 84 to 90 per cent of the theoretical yield.

The whey is heated to boiling, acidified to pH 5.0 with sour whey or acid, and the precipitated protein removed by filtering. After the filtrate has cooled to 33–34° C. (93° F.), 1 lb. of the yeast is added for each 120 gallons of whey, and the fermentation continued at constant temperature until it is complete, usually for about 50 hours. The yeast is removed and the alcohol recovered by distillation. The protein, spent yeast and residues from the still are suitable for feed. The alcohol is of sufficiently good quality to be used for the production of spirit vinegar, as described later.

Riboflavin. Dried whey is fed to chickens not alone for its protein and lactose contents but also for its riboflavin content. During World War II when drying equipment was being used extensively in drying whole and skim milk, the difficulty of producing enough dried whey to satisfy the

needs for riboflavin in feeds was overcome by increasing the riboflavin content of whey before drying by means of a fermentation process (4, 65, 69, 73, 96, 144).

The reaction of raw whey is adjusted to between pH 6.0 and 7.0, and its iron content adjusted to between 1 and 2 parts per million. Five pounds of corn meal and 2 lb. of calcium carbonate are added for each 1,000 lb. of whey and the mixture sterilized by heating under pressure at 250° F. for 15 to 20 minutes. After the whey has been cooled to 100° F., 50 lb. of an active starter of *Clostridium acetobutylicum* (American Type Culture Collection no. 824 or other suitable strain) is added for each 1,000 lb. of whey and fermentation allowed to continue at 86 to 98° F. for 48 hours, or until riboflavin concentration no longer increases. A yield of at least 30 γ of riboflavin per g. of whey can be expected. About 30 per cent of the lactose of the whey is converted during the fermentation to alcohols and acetone. Of these compounds, two-thirds is butyl alcohol, which is of sufficient value to warrant recovery by distillation. It usually is not feasible to recover the small quantities of ethyl alcohol and acetone present.

UTILIZATION OF PRIMARY PRODUCTS

Feed Uses

Fluid, concentrated and dried whey. The use of whey in feeding animals has been mentioned briefly at the beginning of this review. It should be emphasized that condensed and dried whey are the forms most practical to use in feeding pigs and chickens (105, 126), especially those that are being raised on farms distant from cheese factories. Dried whey is considered especially useful in feeding chickens because of the effects of lactose in preventing coccidiosis and the effects of riboflavin on growth of chicks, hatchability of eggs, and prevention of curled toe paralysis. The riboflavin content of whey that is to be dried for chicken feed can be increased advantageously prior to drying by the fermentation procedure outlined in the preceding section.

The concentrated and dried forms of whey usually are fed in mixtures with other feed materials, or the mixing may be done prior to concentration (75, 83). Sweet or soured whey, preferably somewhat concentrated, is recommended as an addition in the making of silage, especially grass silage to which it furnishes nutrients and lactic acid as a preservative (1, 45, 116).

Food Uses

An early record of the benefits to health to be gained through the consumption of whey as a food is found in Hoffmann's "Treatise on the Virtues and Uses of Whey", published in 1761 (44). But, although whey long has been offered as a cure for many illnesses, it is only recently that serious efforts have been made to incorporate it in foods.

The use of whey products in food manufacture affords a much more efficient means for utilization of whey from the standpoint of human nutrition than does feeding to animals followed by consuming the animals as food. The food value of the solids of whey is high and under favorable conditions these solids can effectively transfer milk flavor to foods. It is highly advantageous that whey protein will not coagulate at low temperatures in the presence of fruit acids and that it forms a soft, easily dispersible curd during the heat treatments used in cooking and canning (132).

Foams and emulsifiers. The foaming properties of whey protein have been studied and compared with those of egg albumin by Peter and Bell (90). They found that the stability of foams made from concentrated whey from which part of the lactose has been removed may be increased by neutralization or by the addition of small quantities of tannic acid, saponin, or bisulfites. Beeching and Severn (7) report the preparation of a heat-coagulable foaming agent by the neutralization and filtration of the mother liquor from lactose manufacture. Whey protein foams may be used in many food preparations. However, whey protein cannot be used in place of egg white in certain cakes and custards in which air must be incorporated by whipping and a firm structure set up by heat coagulation. A whey protein whip will not support other ingredients when coagulated by heat.

Sweetened condensed whey can be whipped in 4 minutes to a foam having 200 per cent overrun and a stability of 15 hours (92). This whip, which resembles marshmallow in appearance, is useful for toppings, icings, fruit whips and similar products.

A foaming material for use in non-alcoholic beverages has been prepared by dissolving whey protein in sufficient sodium hydroxide so that the solution has a pH value of 7.0, and then adding an edible acid, such as citric, to bring the reaction to pH 4.0-5.0 (33).

A mixture of 10 lb. of lipoid-free dried whey and 80 lb. of whole egg was found by Clickner (21) to have as good emulsifying properties as pure egg yolk and to be suitable for use in mayonnaise.

Whey drinks. These are made by adding highly flavored fruit or vegetable juices to whey. Whey adds to the nutritive value of a beverage, but it generally does not improve its flavor. A tomato-whey drink is made by mixing 65 per cent tomato juice, 0.4 per cent salt, and 34.6 per cent whey, including enough whey cream to give 2 per cent fat in the finished beverage (133). The reaction should be pH 4.4. The mixture is heated to 140° F., homogenized at 2,500 lb. pressure, canned, and sterilized by heating at 200° F. for 25 minutes. Other vegetable juices or fruit juices may be substituted for tomato juice. These beverages, when freshly mixed and promptly used, have flavor, color, and body superior to those of the canned and sterilized product.

A buttermilk type of beverage may be made by segregating the whey protein in a part of the whey (113). The whey from which the fat has been separated is boiled to coagulate the whey protein, the clear portion (five-sixths of the total) drawn off and discarded, and the remainder homogenized to redisperse the protein. The product contains about 4.1 per cent whey protein, 4.8 per cent lactose, and 0.5 per cent each of ash and fat. It should be possible to prepare a product of approximately this composition by means of high-speed centrifugal separation of boiled cheese whey, as discussed in the section on whey protein.

Soups. For immediate consumption soups can be prepared by using fresh whey in place of water. Soups that are to be canned and sterilized are made more easily with whey than with milk solids. A tomato soup containing whey solids retains the natural tomato acidity and does not contain clots or lumps of protein after heating. A formula for cream style tomato soup follows (132) :

Whey solids, 4 per cent; milk fat, 4 per cent; flour or starch binder, 2.8 per cent; salt, 1 per cent; sugar, 1 per cent; fresh tomato juice, 70 per cent; added water, 17.2 per cent. Warm the mixture to 110° F., homogenize it at 2,500 lb., heat to 176° F., can, and sterilize by heating at 240° F. for 60 minutes without agitation.

Cheese and cheese foods. These may be divided into three classes: (a) whey cheese, (b) whey protein cheese, and (c) process cheese foods.

Whey cheese, known as mysost or primost, has been made for centuries in northern Europe, but the quantity produced in the United States is small. It is made by boiling the whey, generally in an open iron pan 8 to 10 feet in diameter. When it has the consistency of mortar, the hot, pasty mass is placed in tubs in which it is cooled and stirred in order to cause the lactose to form small crystals (27, 102, 122). Primost is packed into greased, wooden, cubical molds to cool and harden.

Albumin cheese was described in 1895 by Babcock (5). Ricotta or Ziger is produced from protein that has been removed from whey by one of the methods described earlier. Sammis (101) states that 5 to 10 per cent of skim or whole milk may be added to the whey before it is heated. The curd is placed in metal hoops, allowed to settle overnight, bandaged and pressed. The cheese may be salted and sold in fresh condition or dried at 110° F.

Whey protein curd recently has been converted into a Roquefort-type cheese (94). Four pounds of curd are recovered from 100 lb. of separated whey and, when pressed, the curd contains about 77 per cent moisture, 16.5 per cent protein, and 2.5 per cent fat. It is probable that whey protein curd can be converted into soft cheeses of other types or into a suitable base for cheese spreads.

Process cheese foods provide one of the largest uses for whey solids. The whey is added to the emulsified cheese mixture in the form of plain condensed or dried whey. Such mixtures contain at least 51 per cent cheese, less than 3 per cent emulsifying salts, organic acids to adjust the reaction to not less than pH 4.5, and a seasoning agent (24). A process cheese food may be made by mixing 93.5 per cent natural cheese, 5 per cent condensed whey containing 65 per cent solids, and 1.5 per cent emulsifying salts, heating with stirring at 165° F., and packaging hot (114).

Bakery products. Products containing whey solids are superior to those containing no milk products, but the products containing whey solids usually are considered inferior to those containing whole or skim milk concentrates. However, Davies (26) has stated that in England “. . . the use of dried whey in bread-making has proved time and again that the size of loaf, texture, taste, and general appeal of the bread are quite equal to that of milk bread.” Any inferiority for this use of whey in comparison with milk is due principally to the relatively high salt and low protein of the whey solids, though the condition of the protein evidently is a factor. Concentrated whey products sometimes impart a salty, acid, or even a bitter taste to bread or cake. There is current interest in new types of whey concentrates developed especially for use in bakery products.

Since Greenbank *et al.* (39) showed that high heat treatment of milk contributed to improvement of bread containing it, whey protein has been suspected of playing an important role in bakery products containing milk. Studies on nitrogen distribution have shown that more than 90 per cent of the whey protein is coagulated when milk is heated above 200° F. for a few minutes (42, 70, 98, 99). This relationship between heat treatment and denaturation of the whey protein has prompted the suggestion that the determination of soluble or undenatured whey protein might be used as a test for the baking quality of dried skim milk (37, 41). In any case, heat treatment of whey destined for use in baked products appears highly desirable.

Processes have been patented for conditioning whey protein (86) and for separating it (54) for use in bread. A dry, comminuted, siftable, water-dispersible shortening composed of particles of fat coated with whey solids has been produced for use in prepared dry mixes and for other bakery purposes (19, 52).

Funder (36), in an extensive study, compared the volumes of water, whey and skim milk breads. For each 100 kg. of flour, he found volume increases over water bread of 6.2 kg. for fluid whey, 13.3 kg. for whey concentrated in the ratio of 2 to 1, and 9.5 kg. for plain skim milk. Several other workers have investigated the use of whey in bread (43, 56, 91, 121).

Whey may be used in a standard bread formula by adding either 3 to 4 lb. of whey solids (as fluid, plain condensed, or dried whey) or 7 to 10 lb. of sweetened condensed whey per 100 lb. of flour. The sugar and water in the formula should be adjusted to compensate for the addition of these ingredients in the whey.

Whey helps in producing a cake-like texture in sweet goods, and it may be added to conventional formulas for cakes and cookies (82, 136). A canned pudding has been developed in which as much as 22 per cent of the solids are whey solids (13).

Candies. Such types of candies as fudge, caramel and taffy can be made with a whey solids content of 14 to 40 per cent (55, 134, 135). Plain condensed, sweetened condensed, or dried whey may be used as a source of whey solids. Sweet rennet-type whey is preferred to neutralized acid whey because of its superior flavor. Whey is especially useful in fudge; the lactose on crystallizing contributes to the desired grainy texture. Whey caramels should be fortified with casein-containing milk solids in order to produce the characteristic chewy body. Whipped sweetened condensed whey may be used to incorporate air in special types of candy. A formula for whey fudge follows (134):

Sweetened condensed whey, 43 per cent; sugar, 11 per cent; corn sirup, 9 per cent; invert sirup, 3 per cent; butterfat, 2.5 per cent; chocolate, 6 per cent; fondant, 20 per cent; powdered lactose, 0.1 per cent; nuts (optional), 5.4 per cent; vanilla to flavor. Cook (with stirring) the condensed whey, sugar, invert sirup, and half the corn sirup. The butterfat is added as cream or butter after the sirup has been partly boiled down. Cook to 248° F. Cool 25 or 30° or transfer to smaller pouring kettles, add the remaining corn sirup, the fondant and chocolate, and stir well for several minutes. Add the powdered lactose, flavoring and nuts. Stir. Pour into wooden forms.

Spirit vinegar. It may be made from the alcohol produced by fermentation of whey by the procedure described earlier. A simple distillation of the fermented whey will yield a dilute alcohol suitable for conversion to vinegar. The dilute alcohol is allowed to trickle over beech shavings or birch twigs impregnated with the acetic-acid-producing organism. A current of air passing upward through the vinegar converter accelerates the fermentation.

Food acidulant. Colorless 50 per cent lactic acid is used as a food acidulant in sherbets and bottled beverages and as a preservative and firming agent for pickles. The production of edible lactic acid from crude acid is a highly technical chemical process (17).

Whey butter. This is made from whey cream in accordance with usual buttermaking procedures. The low ash and the absence of casein make

possible rapid churning. Hence, a butter of good body and texture can be produced by churning at a lower temperature than customarily is employed in churning cream from whole milk. The churning time of cream from whole milk can be shortened somewhat by adding whey cream.

Pharmaceutical Uses

Whey furnishes raw material for the preparation of several important pharmaceuticals, such as protein hydrolyzates and penicillin. Certain other products derived wholly or in part from whey and sold over the pharmaceutical counter in drug stores might properly be classed as foods, such as lactose and infant foods. The use of lactose as a basis for pills definitely is a pharmaceutical use.

Lactose. This often is added to infant foods based on cows' milk for the purpose of making the composition of the food more nearly like that of human milk. High grade technical or USP lactose is added to modified cows' milk in sufficient quantity so that it has a concentration of carbohydrate that may be as great as 52 per cent of the solids of the milk. Such infant foods generally are either dried or canned and sterilized. Lactose also is sold for use in feeding formulas prescribed for preparation in the home. Because the common or *alpha* lactose is only slowly soluble, it has been found more convenient to use the more rapidly soluble *beta* form of this sugar. The resulting solution is the equilibrium mixture of the two forms, whether *alpha* or *beta* lactose is used in making the solution.

Riboflavin concentrates. Riboflavin and other materials can be purified and concentrated by adsorption on lactose (63, 64, 84). By suitable choice of degree of super-saturation of the lactose, concentration of riboflavin, and working temperatures, adsorbates containing approximately 300 γ of riboflavin per g. of lactose can be produced. It is possible to obtain even greater concentrations if excessive time is allowed for the crystallization of the sugar. The mother liquor of lactose manufacture may be used for the preparation of riboflavin adsorbate. After the removal of crude lactose that has crystallized at 140° F., the filtrate is cooled to between 40 and 50° F. and seeded with lactose crystals. A second crop of lactose crystallizes slowly over a period of 24 hours and contains about 100 γ of riboflavin per g. of sugar. By recrystallization of this crude adsorbate, the concentration of riboflavin can be increased to about 300 γ per g. of lactose.

Penicillin. Since 1943, several million lb. of lactose have been used each year as a component of the nutrient mixture in which penicillin is produced. Lactose acts to ensure higher yields than are obtained when it is not used, but it is not essential in the process. The possibilities of substitution

of other carbohydrates for lactose, of synthetic production of penicillin, and of the discovery of other antibiotics that will supersede penicillin make it seem unlikely that lactose long will be used for this purpose.

Hydrolyzed lactose. Lactose hydrolysis, which produces a mixture of glucose and galactose, offers the possibility of preparing a sweeter, more soluble form of carbohydrate from lactose for food use. Impure lactose, or the lactose in whey, when hydrolyzed by acid, yields a product which has a disagreeable taste and is contaminated with whey salts, humin and other products of protein hydrolysis.

Ramsdell and Webb (93) in hydrolyzing pure lactose found that there was a gradual destruction of glucose as it was formed. They found the optimal procedure to be as follows:

Heat 2,100 g. of pure lactose, 49 g. of N hydrochloric acid and 4,851 g. of water to 297° F. in a pressure kettle. Allow 60 minutes to reach 297° and hold 5 minutes. Add carbon black, filter, concentrate to 60 per cent solids, and adjust the reaction to pH 5.0.

Glucose and galactose to the amount of 93 per cent of the theoretical are obtained. The solubility of a mixture of equal parts of both is 42 per cent. The maximal concentration of a mixture of the two hexoses soluble at 77° F. is 58.3 per cent and consists of 49.8 per cent glucose and 8.5 per cent galactose. A process for making hydrolyzed lactose caramel has been described (79).

Enzymic hydrolysis of lactose in whey and other dairy products would be the simplest method of reducing the concentration of lactose and increasing the sweetness of the product. The use of an enzyme obtained from kefir grains to treat milk products to be used in ice cream has been suggested by Turnbow (127). However, no adequate source of a lactase has been available. Browne and Webb (14) investigated a number of possible sources, but were unable to obtain uniformly active lactase preparations suitable for commercial use. Current work in several laboratories, however, seems likely to result in the preparation of an enzyme of adequate strength in commercial quantities.

Hydrolyzed protein. Protein hydrolyzates usually are prepared by pharmaceutical companies, although several of the large dairy organizations have manufactured the hydrolyzate from whey protein. The flavor of the hydrolyzate derived from whey protein is said to be less objectionable than that derived from casein. Protein may be hydrolyzed by acid, by alkali or by enzymes. The reaction is complete when all the peptide linkages are broken. The rate of hydrolysis is that of a second-order reaction.

Sahyun (100) has reviewed the factors concerned with protein hydrolysis. Acid hydrolysis may be conducted with any of several acids, although

sulfuric generally is preferred. Tryptophane is destroyed during acid hydrolysis. The following directions for hydrolysis of protein by acid and by enzyme were given by Sahyun (100):

Two kilograms of protein is mixed with 14 l. of 5 N (25 per cent) sulfuric acid, the mixture autoclaved at 248° F. for 16 hours, limed to pH 10, filtered, and the residual calcium and sulfate ions removed. The amino acid preparation then is concentrated, and sterilized or dried.

There are numerous proteolytic enzymes capable of hydrolyzing proteins. Some of the amino acids are liberated sooner than others, but the degradation follows the general pattern: Protein → proteoses → peptones → peptides → amino acids.

To hydrolyze 100 g. of protein, it is mixed with 700 cc. of water, 10 cc. chloroform, 0.5 g. pancreas extract (trypsin), and sufficient 5 N (20 per cent) sodium hydroxide to produce a reaction of pH 8. The mixture is incubated under controlled conditions for 8 to 12 days, heated, filtered, concentrated and dried. (100)

When proteins are hydrolyzed by alkali, no humin is formed, as is the case in acid hydrolysis. However, the amino acids, with the exception of glycine, are racemized, which is objectionable, since not all of the racemic amino acids are utilized in animal metabolism.

Therapeutic products. Products consisting chiefly of the protein and mineral constituents of whey have been prepared by patented processes. One method is to precipitate the protein-mineral complex with alkali, separate it, wash or reprecipitate it to free it from any objectionable protein decomposition products, and finally dry it (95, 129). Another method is to precipitate electrolytically, filter, and wash and dry the precipitate. A product thus is obtained containing approximately 18 per cent calcium, 6 per cent phosphorus and 20 per cent protein (22). Another therapeutic product consists of whey, an edestin-calcium solution derived from hempseed, and magnesium sulfate (112).

Chemical Uses

Lactic acid. This is used industrially in leather manufacture (142). The highly colored, crude grades, marketed in 22 or 44 per cent concentration, are used in diluted condition to neutralize the lime in limed hides. The requirements for this purpose of a weak acid forming a soluble calcium salt are satisfied by lactic acid. Sodium lactate solutions resemble glycerol in consistency and are used as substitutes for it in textile printing and in paper-making. Ethyl, butyl and other lactate esters have use as solvents and plasticizers. Lactic esters can be used as starting materials for the production of the industrially important acrylates.

Butyl alcohol. This is a by-product of the fermentation producing riboflavin, and its esters are useful as solvents.

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EFFECT OF SEASON, BREED AND SPECIES OF RUMINANTS ON THE VITAMIN A POTENCY OF BUTTERFAT

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Under European and American conditions, the vitamin A potency of butter from cows generally is found to be maximum in summer and minimum in winter (3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 19). It also has been reported that the variation in potency is influenced but little by the stage of lactation (6, 9, 18). Since Indian climate and feeding practices vary markedly from those in Europe and America, a study of the seasonal variation in vitamin A and some of the other constituents of milk and butterfat was considered desirable.

In previous reports from this laboratory (16, 17) the effect of various levels of carotene ingestion on the vitamin A potency of milk and butterfat of Haryana cows was studied. In order to make a comparative study of the vitamin A potency of butterfat from different breeds and from other species of ruminants, further work subsequently was carried out and some of the results are presented in this paper.

EXPERIMENTAL PROCEDURE

The Institute dairy herd consisting of from 23 to 33 Haryana animals received different types of roughages, depending upon the season of the year, and a concentrate mixture (wheat bran 40 parts, gram husk 20 parts, ground nut cake 20 parts, rape cake 10 parts, and gram chuni 10 parts) at the rate of 1.5 lb. for maintenance and 1 lb. for each 2 lb. of milk produced. The animals, in addition, received 1 oz. of iodized salt and 1 oz. of bone meal per head daily.

The investigation lasted from April 1, 1941, to July 15, 1942, during which time fortnightly analyses were made to determine the carotene content of the feeds and the fat, carotene and vitamin A contents of the composite herd milk sample collected over a 3-day period from two milkings.

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a day. The milk samples were analyzed also for total solids, protein, ash, calcium and phosphorus from the middle of November, 1941, until the conclusion of the experiment. Butter samples were prepared during typical drought periods (May and December) and green-feeding periods (February and July) in both winter and summer and analyzed for the Reichert-Meissl, Polenske, saponification and iodine values. The methods of analysis were the same as those previously reported (16).

To study the influence of breed and species, short-time experiments were conducted with Sahiwal heifers, goats and water buffaloes. Three Sahiwal first-calf heifers, each weighing approximately 688 lb., were fed berseem *ad libitum* in addition to the regular dairy grain mixture for a period of 19 days. The average consumption of berseem was 59 lb. and that of carotene was 1,009 mg. per day. Four goats, each having an average body weight of 67 lb., received berseem in addition to a concentrate for 18 days. The average daily consumption of berseem was 3,292 g. and the ingestion of carotene amounted to 108 mg. Two water buffaloes, each weighing approximately 865 lb., received 50 lb. of berseem daily. The level of feeding was not *ad libitum*, owing to the shortage of berseem, and the experimental period was only 11 days. The daily consumption of carotene was 785 mg. per animal. All of the milk samples used in this work were collected from two milkings a day during the last 3 days of the experimental period and analyzed for carotene, vitamin A, fat, solids-not-fat, protein and ash.

In calculating the total vitamin A potency of the butterfat, 0.6 μ g. of the carotene and 0.25 μ g. of the vitamin A were each taken as equal to 1 international unit of vitamin A (1).

RESULTS

Variations in vitamin A potency of butterfat. Table 1 presents the composite data for carotene, vitamin A, and total vitamin A potency of butterfat for each month of the experiment and the level of carotene ingestion for each corresponding month. In general, the maximum total vitamin A potency was reached during July, August and September (24,734 I.U. per lb.) and then diminished gradually through January. The average minimum value (16,093 I.U.) was obtained in November, December and January and then the potency began to rise, reaching the maximum value in March (24,861 I.U.). During April, May and June, the potency declined rapidly but in July the potency increased markedly. The maximum increase in total vitamin A potency was 55 per cent during the experimental period. The variations observed in total vitamin A potency were not due entirely to carotene activity or vitamin A activity alone but to a combination of both. The periodic fluctuations in carotene and vitamin A in butterfat can be correlated with the level of carotene ingestion subsequent to changing the type of fodders. On the microgram basis, the maximum and

minimum values reported in this paper are in agreement with those previously reported by Ray Sarkar and Sen (16) in experiments with cows under intensive green-feeding conditions. From the data on carotene ingestion (table 1) it has been calculated that a daily intake of about 45 lb. of average green fodder per cow will maintain the maximum vitamin A potency throughout the year (16).

TABLE 1

Seasonal variation in the vitamin A potency of butterfat of Haryana cows

Month	Av. daily carotene intake per cow	Vitamin A potency of butterfat		
		Carotene	Vitamin A	Total
(1941)	(I.U.)	(I.U. per lb.)		
April	541,800	1,582	15,155	16,737
May	521,400	1,823	16,281	18,104
June	695,500	2,291	18,844	21,135
July	990,100	2,483	22,259	24,742
Aug.	1,495,300	2,712	22,126	24,838
Sept.	1,369,600	2,833	21,790	24,623
Oct.	507,000	2,389	18,486	20,875
Nov.	260,200	1,790	14,210	16,000
Dec.	210,700	908	15,301	16,209
(1942)				
Jan.	450,400	1,269	14,802	16,071
Feb.	1,268,500	2,671	19,786	22,457
March	850,400	2,814	22,047	24,861
April	379,500	1,793	19,315	21,108
May	154,800	1,375	16,386	17,761
June	139,800	1,378	16,286	17,664
July	1,625,400	2,756	18,770	21,526

Table 2 shows the effects of carotene-poor and carotene-rich rations on the carotene and vitamin A contents and on the total vitamin A potency of butterfat of cows at different stages of lactation. The vitamin A potency of butterfat in the post-colostral period remains practically unchanged with the progress of lactation, provided the same level of carotene ingestion is maintained throughout the lactation period. This observation is in conformity with that of Treichler *et al.* (18), Gillam *et al.* (9), and Brown *et al.* (6). These results indicate that the stage of lactation has little effect on the seasonal variation in the vitamin A potency of butterfat.

Seasonal effect on the quality of butterfat. A limited number of chemical constants were determined on the butterfat samples prepared at different seasons of the year. These results are summarized in table 3. The data indicate that within the same season, the iodine values increase when green fodder is included in the ration (samples 2 and 4), whereas the Polenske values increase during the summer months (samples 3 and 4). Apparently neither the feeding of green fodder nor season had any appreciable effect on the Reichert-Meissl or saponification values. The Reichert-Meissl num-

ber, however, seems to be significantly lower and the iodine number significantly higher than that reported for western butterfat. The Polenske value tends to be lower but the saponification number is the same as that reported for western butterfat. These observations, however, are tentative and must be confirmed by future experiments.

TABLE 2
Effect of stage of lactation on carotene and vitamin A contents of the butterfat of Haryana cows

No. of cows	Month of lactation	Av. daily milk yield	Av. fat	Vitamin A potency of butterfat		
				Carotene	Vitamin A	Total
		(lb.)	(%)	(I. U. per lb.)		
Cows receiving carotene-poor rations						
5	1	12.0	5.2	785	14,876	15,661
2	2	12.5	4.8	708	14,876	15,584
5	3	11.4	4.8	785	15,242	16,027
2	4	15.5	4.7	729	14,144	14,873
3	6	9.7	5.2	652	15,775	16,427
1	7	4.0	4.5	776	15,232	16,008
	Weighted av.			748	15,066	15,814
Cows receiving carotene-rich rations						
5	3	11.8	4.5	2,712	21,660	24,372
4	4	10.3	4.5	2,349	21,717	24,066
5	5	9.0	4.4	2,590	21,548	24,138
4	6	7.5	4.9	2,692	21,484	24,176
2	7	9.0	4.9	2,481	21,412	23,893
1	8	9.0	4.5	2,242	21,791	24,033
	Weighted av.			2,566	21,594	24,160

Variations in other milk constituents. The variations in the quality of milk at various seasons as measured by differences in some of the main constituents are shown in table 4. The maximum, minimum, and average values obtained during the experimental period were 5.6, 4.5, and 4.9 per

TABLE 3
Chemical constants of the butterfat from herd milk at different seasons

Sample no.	Month	Reichert-Meissl value	Polenske value	Saponification value	Iodine value
1	Dec.	22.0	1.1	226.0	36.0
2	Feb.	23.5	1.6	226.2	40.0
3	May	24.0	2.2	227.0	37.0
4	July	23.4	2.2	226.8	39.3

cent for fat; 8.97, 8.62, and 8.75 per cent for solids-not-fat; 3.70, 3.43, and 3.53 per cent for protein; 0.808, 0.729, and 0.771 per cent for ash; 0.150, 0.125, and 0.138 per cent for calcium; and 0.107, 0.091, and 0.100 per cent for phosphorus, respectively. However, the mean fat percentage for the

15.5-month experimental period was 4.8. The fat content tended to remain at a higher level during November, December and January, when a seasonal decline in milk production occurs due to the marked drop in temperature.

TABLE 4

Composition of herd milk at different seasons of the year (Hariana cows)

Period	Fat	S.N.F.	Protein	Ash	Ca	P
(1941)	(%)	(%)	(%)	(%)	(%)	(%)
Nov. 16-30	5.6	8.78	3.50	0.788	0.150	0.104
Dec. 1-15	5.4	8.68	3.45	0.729	0.143	0.096
16-31	5.1	8.87	3.43	0.793	0.138	0.098
(1942)						
Jan. 1-15	5.2	8.97	3.70	0.778	0.138	0.103
16-31	5.2	8.84	3.61	0.778	0.148	0.097
Feb. 1-15	4.8	8.70	3.56	0.780	0.140	0.107
16-28	4.7	8.62	3.48	0.769	0.133	0.105
March 1-15	4.8	8.77	3.60	0.780	0.143	0.105
16-31	4.5	8.78	3.52	0.769	0.143	0.107
April 1-15	4.6	8.70	3.45	0.760	0.140	0.100
16-30	5.0	8.64	3.48	0.808	0.145	0.099
May 1-15	4.7	8.78	3.50	0.769	0.128	0.091
16-31	4.8	8.64	3.48	0.760	0.125	0.093
June 1-15	5.0	8.73	3.52	0.740	0.143	0.105
16-30	4.7	8.70	3.47	0.788	0.140	0.098
July 1-15	5.0	8.85	3.60	0.770	0.129	0.102
16-31	4.7	8.72	3.67	0.747	0.128	0.091
Av.	4.9	8.75	3.53	0.771	0.138	0.100

There also were differences of 20.0 and 17.6 per cent, respectively, between the lowest and highest calcium and phosphorus values. Apart from the relatively wide percentage differences in fat, calcium and phosphorus, the changes in the other constituents were slight.

TABLE 5

The various constituents in milk and the carotene and vitamin A contents of butterfat from heifers, goats and buffaloes

	Heifers	Goats	Buffaloes
Milk yield	10.6 lb.	383.5 ml.	16.2 lb.
Fat (%)	6.5	5.1	7.9
S.N.F. (%)	8.59	9.28	9.90
Protein (%)	3.73	4.54	4.03
Ash (%)	0.750	0.897	0.856
Carotene (I.U.) ^a	2,782	Trace	Trace
Vitamin A (I.U.) ^a	23,050	26,498	17,879
Total potency (I.U.) ^a	25,832		

^a Per lb. of butterfat.

Constituents in milk and butterfat of heifers, goats and buffaloes. The data pertaining to the amounts of various constituents in milk and the carotene and vitamin A in butterfat from the three species are shown in table 5. Although the number of animals is small, it may be said that the

various species do differ in regard to the secretion of the various constituents in milk. This observation is not new. The interesting fact is the trace of carotene in the butterfat from goats and buffaloes, an amount which is not measurable quantitatively by the usual colorimetric method. Considerable individual variation in the vitamin A content also was noted in the case of each species. So far as total vitamin A potency of butterfat is concerned, goats and cows are almost equally efficient. It was observed subsequently in the case of the goats that the same potency could be attained even at a level of 60 per cent of the original carotene ingestion. The vitamin A potency of the butterfat of the buffalo was comparatively low. A true comparison could not be made because the level of carotene ingestion was not high enough owing to the shortage of green fodder. Subsequent studies with other animals under heavy green-fodder feeding have shown, however, that buffalo butterfat might contain as much as 20,480 I.U. per lb. as compared to 25,000–26,000 I.U. in the case of butterfat from cows and goats.

A comparison of the figures previously obtained for carotene and vitamin A in the butterfat from Haryana cows shows that the vitamin A potency is the same for the Sahiwal and Haryana breeds (16) when calculated on the same basis. The concentration of vitamin A in the butterfat of cows so far examined compares fairly well with that found for some western breeds (2, 4, 9), but the picture is different with respect to carotene. The two Indian breeds studied so far definitely secrete less carotene in butterfat. It is difficult to say without further experimentation where this physiological difference lies. In view of the lack of knowledge regarding the fate of carotene in the rumen, much importance has not been given to the value of about 70 per cent apparent fecal excretion in the case of cows and goats, as determined incidentally. If the rest of the carotene were absorbed, then the recovery of absorbed carotene as carotene and vitamin A in milk would amount to about 3.5 per cent. This value is practically the same for the heifers and goats under comparable feeding conditions. There is very little carotene in the plasma of goats as compared to 0.891 mg. per cent in the plasma of the Sahiwal heifers. On the other hand, the blood plasma levels of vitamin A were 0.170 mg. and 0.157 mg. per cent, respectively, for heifers and for goats. Buffalo blood was not examined in this connection. Goats and buffaloes possibly convert more of the absorbed carotene into vitamin A, which is readily transmitted into the milk.

SUMMARY

The seasonal variations in vitamin A potency of butterfat and other constituents in herd milk of Haryana cows have been investigated. The vitamin A potency varied with the level of carotene intake. The potency was maximal in the monsoon periods (July, August and September) when the cows were getting sufficient carotene from grazing and again during the

winter months (February and March) when large quantities of cultivated fodders were available. The average maximum total potency approximated 24,972 I.U. per lb., of which 2,700 I.U. were due to carotene. The average minimum potency of 16,093 I.U., of which 1,322 I.U. were due to carotene, was obtained in November, December and January, when very little green feed was available. The maximum variations in carotene, vitamin A, and total vitamin A potency were 212.0, 56.6, and 55.0 per cent, respectively. The vitamin A potency was not influenced by the stage of lactation.

Except for fat content, which was about 25 per cent higher in November, December and January, and for calcium and phosphorus, the solids-not-fat, protein and ash content of the milk remained unchanged throughout the experimental period.

The Polenske values of the butterfat were higher in summer than in winter but the Reichert-Meissl and saponification values did not show any seasonal change. The saponification value was the same as that reported in the literature for American butterfat, but the Reichert-Meissl number was approximately 20 per cent lower and the iodine number was about 10 per cent higher. The inclusion of green feed in the ration tended to increase the iodine value.

There was practically no difference in the carotene and vitamin A contents of butterfat from the cows of the Hariana and Sahiwal breeds. Although butterfat from goats contained only traces of carotene, the vitamin A content was as high as the total potency in the butterfat from cows. Buffalo butterfat examined in this investigation resembled that of goats in respect to carotene, but the vitamin A content was comparatively low.

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PARTURIENT PARESIS. II. THE EFFECT OF PARTIAL VERSUS COMPLETE MILKING UPON THE TOTAL BLOOD SERUM CALCIUM OF DAIRY COWS AT PARTURITION¹

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A common practice among dairymen is to partially milk cows for a few days following parturition, because it is believed to reduce the incidence of parturient paresis or milk fever. Several workers (2, 3, 6) have demonstrated that air inflation of the udders of cows with parturient paresis results in an increase in the total serum calcium and generally brings about recovery. When air pressures equal to 25 to 40 mm. Hg were maintained in the udder, inhibition of milk secretion was almost complete (4, 5). The efficacy of the air inflation treatment for parturient paresis has been attributed to resorption of milk or cessation of milk secretion caused by increased intramammary pressure, thereby preventing a further uptake of calcium by the mammary gland. As a result of these studies, the belief has become prevalent that the incidence of parturient paresis could be lowered if some milk was left in the udder to maintain pressure. This study was undertaken to learn the effect of complete milking immediately following parturition upon the incidence of parturient paresis and total blood serum calcium.

EXPERIMENTAL PROCEDURE

Cows used were of the Holstein, Guernsey and Jersey breeds. They were divided into two groups by alternating cows within each breed. The cows in the partially milked group were managed in the conventional manner by permitting the calves to remain with the cows for 3 days following calving. About two-thirds of the milk was removed from the udders of this group beginning the day after calving. Complete milking began the fourth day subsequent to calving.

Calves were removed from the dams in the completely milked group before nursing, and the cows were milked completely with the aid of an intravenous injection of 10 I.U. of oxytocin from 1 to 3 hours after calving. The cows then went on the regular two-time milking schedule and were in-

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TABLE 1
Daily levels of total blood serum before and after parturition

Total blood serum calcium—mg. %													
Cow no.	Days prepartum					Day of parturition	Days postpartum					Date of parturition	Remarks
	5	4	3	2	1		1	2	3	4	5		
Jerseys (completely milked group)													
711	10.1	11.2	10.9	10.2	10.9	9.9	—	9.5	10.4	10.0	10.3	6-13-45	Mild symptoms of milk fever
703					10.6	9.6	6.0	8.1	9.5	8.7	9.5	6-23-45	
667		9.4	9.8	9.8	9.7	5.9	7.9	10.6	10.3	10.3	—	6-27-45	Mild symptoms of milk fever
713	9.5	9.6	9.4	9.3	8.1	7.6	9.0	9.7	10.2	10.3	10.4	8-25-45	
B61			9.8	9.9	9.5	5.0	5.8	8.5	9.0	9.5	9.3	2-22-46	Mild symptoms of milk fever
B58				9.9	9.1	4.0	5.6	3.7	7.9	9.9	10.1	3-21-46	
705			10.0	9.9	9.8	6.8	6.3	7.0	7.2	9.4	9.9	8-3-46	Milk fever on 3-21-46
696	10.2	9.9	9.9	10.4	10.2	6.1	4.1	8.4	9.0	9.5	10.2	9-15-46	
731			10.7	10.4	10.1	9.7	10.1	10.4	10.6	—	—	9-23-46	Milk fever on 9-16-46
729	10.7	10.8	10.8	10.0	10.5	9.7	10.1	10.8	10.8	—	—	11-22-46	
B57	9.8	10.4	10.2	10.1	—	8.4 ^b	10.9	7.6	8.0	9.6	8.9	4-14-47	Milk fever on 4-14-47
Jerseys (partially milked group)													
705	9.9	10.2	10.1	10.4	11.3	8.2	4.2	6.4	8.2	9.3	10.3	6-26-45	Milk fever on 6-27-45 Milk fever 8-21-45, died 8-23-45
671	10.0	10.6	10.2	10.2	9.3	7.4	4.7	7.6	14.4 ^b	—	—	8-20-45	
690			11.3	10.3	10.6	4.4	6.6	10.9	11.4	11.6	—	10-5-45	Milk fever on 10-5-45
731	10.5	9.2	11.6	10.6	10.9	10.3	10.3	10.2	10.6	10.6	10.4	10-22-45	
B57					9.3	6.0	4.1	7.2	10.4	11.3	11.6	12-2-45	Milk fever on 12-3-45
B63					9.2	4.7	7.8	11.2	10.7	10.9	10.7	3-24-46	
711	9.7	9.7	10.2	9.8	9.8	8.4	7.6	9.6	10.3	10.9	10.7	8-8-46	Milk fever on 3-24-46
713					9.8	—	8.7	8.5	5.9	6.1	7.9	8-31-46	
												9-18-46	Milk fever 8-31-46, died 9-18-46
703	10.2	10.7	10.9	10.7	8.5	4.0	8.0	10.8	10.4	10.6	10.2	9-15-46	
720				10.5	8.4	6.7	9.6	10.4	10.7	10.7	—	12-9-46	Milk fever on 9-15-46
B61			10.4	10.8	10.3	4.9	6.1	7.5	10.8	10.2	10.0	4-10-47	

^a Either blood sample was not drawn or was destroyed accidentally.

^b Treated for milk fever before blood sample was taken.

jected intravenously with oxytocin at each milking for 5 days subsequent to calving.

TABLE 2
Average total blood serum calcium

	Total blood serum calcium—mg. %											Remarks
	Days prepartum					Day of parturition	Days postpartum					
	5	4	3	2	1		1	2	3	4	5	
<i>Jerseys</i>												
Completely milked group												
Av. 11 cows	10.1	10.2	10.2	10.0	9.9	7.5	7.6	8.6	9.4	9.7	9.8	3 cases milk fever
Partially milked group												
Av. 11 cows	10.1	10.1	10.7	10.4	9.8	6.5	7.1	9.1	10.3	10.2	10.2	8 cases milk fever
Both groups												
Av. 22 cows	10.1	10.2	10.4	10.2	9.8	7.0	7.3	8.8	9.8	10.0	10.0	11 cases milk fever
<i>Guernseys</i>												
Completely milked group												
Av. 9 cows	10.3	10.3	10.2	10.4	10.1	8.8	9.2	9.7	9.6	10.0	10.1	
Partially milked group												
Av. 11 cows	9.9	10.4	10.2	10.0	9.9	9.0	9.3	9.4	9.9	9.8	10.0	
Both groups												
Av. 20 cows	10.1	10.3	10.2	10.2	10.0	8.9	9.3	9.5	9.8	9.9	10.0	
<i>Holsteins</i>												
Completely milked group												
Av. 7 cows	10.1	10.5	10.4	10.5	9.8	9.0	8.4	9.3	10.1	9.8	10.3	
Partially milked group												
Av. 7 cows	11.1	10.2	10.5	10.3	10.1	9.5	9.7	9.9	9.9	10.2	9.9	
Both groups												
Av. 14 cows	10.4	10.4	10.5	10.4	10.0	9.2	9.1	9.6	10.0	10.0	10.1	
<i>Summary of all breeds</i>												
Av. 27 completely milked cows	10.2	10.3	10.2	10.3	9.9	8.3	8.4	9.1	9.6	9.8	10.0	3 cases milk fever
Av. 29 partially milked cows	10.2	10.2	10.4	10.2	9.9	8.2	8.5	9.4	10.1	10.1	10.0	8 cases milk fever

Venous blood samples were taken daily for 5 days previous to the anticipated day of parturition and for the first 5 days subsequent to calving. Blood samples were drawn within an hour of the same time daily. Total

blood serum calcium was determined by a modification of the Clark-Collip method (1).

The experiment ran from June, 1945, until May, 1947, and included a total of 56 cows, of which 29 were in the partially milked group and 27 in the completely milked group. All cows of the three breeds mentioned freshening in the experimental herd were included, with the exception of first-calf heifers, which rarely if ever have parturient paresis.

A case was not diagnosed as parturient paresis or milk fever unless the cow was "down" or in a coma with typical symptoms. Two cows in the completely milked group showed mild symptoms of milk fever but never went "down" and recovered without treatment.

RESULTS

Data for the Jerseys of both groups are presented in table 1. The average figures of total blood serum calcium for both groups of all breeds, as well as a summary of all breeds, are presented in table 2. The complete data for the other breeds are not presented, as all cases of milk fever occurred within the Jersey breed. The over-all incidence of the disease was 19.6 per cent, with eight cases of milk fever occurring in the partially milked group and three cases in the completely milked group. The Jersey breed had an incidence of 50 per cent, with 27.3 per cent in the completely milked group and 72.7 per cent in the partially milked group. Two of the Jersey cows (671 and 713, while in the partially milked group) died, although calcium treatment was administered. Cow no. 713 developed a case of severe ketosis, for which treatment proved ineffective, and this was the ultimate cause of her death.

Total blood serum calcium levels exhibited a characteristic drop on the day of calving, with a gradual return to normal by the fourth or fifth day subsequent to calving. The greatest drop was evident in the Jersey breed, with little difference in the averages of the Holstein and Guernsey breeds. The total blood serum calcium levels of the two groups closely parallel each other.

SUMMARY

The complete milking of cows immediately following parturition did not increase the incidence of parturient paresis or was the average total serum calcium greatly different for the two groups.

Determinations for total blood serum calcium were made on samples of blood collected daily from 27 cows of the completely milked group and 29 cows of the partially milked group for 5 days prior to the anticipated day of parturition and for 5 days following calving.

Two cows showed mild symptoms and three had parturient paresis in the completely milked group. Eight cows in the partially milked group had

parturient paresis. All cases of parturient paresis occurred in the Jersey breed.

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THE VALUE OF HYPOCHLORITE AND QUATERNARY AMMONIUM COMPOUNDS, WHEN USED IN UDDER WASHES, IN REDUCING THE PLATE COUNT OF MILK^{1,2}

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Various bactericidal agents now are in general use in solutions for washing the udders of cows prior to milking. Comparatively little information is recorded regarding their value in reducing the plate count of the milk produced. Scales and Kemp (6) reported that 2 minutes was too short a period for chlorine to yield dependable sterility. Other workers, Bryan *et al.* (2) and Waugh *et al.* (9), have shown that no viable organisms were present in recommended concentrations of chlorine. Spurgeon *et al.* (8) compared the bactericidal activity of hypochlorites and quaternary ammonium compounds. When applied to teats which had been inoculated with a suspension of *Streptococcus agalactiae*, these germicidal solutions did not eliminate all the organisms present but did destroy 90 per cent of those that would have remained after ordinary rinsing of teats with non-germicidal solutions. Mueller *et al.* (5) found that approximately 0.3 per cent of cow feces or nonfat milk solids produced a significant decrease in germicidal potency of a 200 p.p.m. quaternary ammonium solution. Keith and Reaves (4) washed udders with quaternary ammonium compounds, chlorine, and plain water. They believe the quaternary ammonium compounds are effective sanitizing agents. Byers and Ewalt (3) reported a reduction of 34.2 per cent in the plate count of milk produced when the udders of cows were washed with chlorine solutions.

Definite information relative to the value of solutions of these substances in washing the udders of dairy cows previous to milking apparently is needed.

EXPERIMENTAL PROCEDURE

Four groups of five cows, each as similar as possible on the basis of level of milk production, age, and stage of lactation, were selected. All were fed and managed alike. Their udders were washed with clean water preparations as follows: Water alone, 200 p.p.m. chlorine, 400 p.p.m. chlorine, 200 p.p.m. quaternary ammonium compound, and 400 p.p.m. quaternary am-

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monium compound. A generally used commercial sodium hypochlorite powder was used for preparing the chlorine solution. Similarly, a widely used commercial preparation containing 10 per cent alkyl-dimethyl benzyl-ammonium chlorides of high molecular weight was used as a source of quaternary ammonium compounds. Two gallons of each of these solutions were prepared at a temperature of 125° F. just prior to milking, using amounts of the bactericides in accordance with manufacturers' directions. A clean turkish towel was used for each solution. Washing was done throughout by the same person. The udder and flanks of the cow were scrubbed thoroughly with the towel, which had been removed from the solution and folded wet. The cloth then was rinsed in the solution, wrung dry, and used to wipe the udder. Only one cow was washed with each water preparation. Two streams of milk were removed from each teat into a strip cup. The milking machines were attached 1 minute after the washing was begun.

Previous to each milking time the milking machines were taken apart and scrubbed thoroughly with a detergent. They then were rinsed in clear, warm water, reassembled, and the complete unit autoclaved at 15 lb. pressure for 20 minutes. Before autoclaving, each teat cup was covered with paper foil which was not removed until the machine was to be attached to the udder. Milking was done by the same persons and at the same time each evening. All five cows were milked simultaneously. Each cow was milked dry and machine stripped only.

When the machine was removed from the cow, a sample of the milk was obtained from the pail with a sterile milk thief and placed in a sterile sample bottle. The samples were iced until plated. They were transported to the laboratory and plated on tryptone-glucose-extract-milk agar within 0.5 hour. Plating and counting were done according to Standard Methods for the Examination of Dairy Products (1). This procedure was repeated 5 days with treatments randomized in a Latin square design so that no cow received the same treatment more than once. Use of the five machines was randomized so that the same machine was used only once on each cow and only once with each treatment during the individual trial.

A separate Latin square design and different cows were used for each of the first four trials. This experiment was conducted on only the evening milking. During the last period, it seemed desirable to determine the difference in response, if any, between evening and morning milking. This will be designated as Trial V, which followed the same Latin square design and used the same cows as Trial IV and was run on the mornings of the same days.

RESULTS

During the course of Trial I, one cow suffered an attack of acute mastitis. In 2 days, the bacteria per ml. in her milk increased from around 1,000 to

70,000. On two occasions the milker allowed the teat cups to touch the bedding as he was attaching the machine. This resulted in visible sawdust in the milk and exceptionally high plate counts. The results on this trial are shown in table 1. An analysis of variance run on the data showed no significant difference between treatments, possibly due to the small number of observations.

TABLE 1
The plate counts of milk obtained in trial 1^a

Cow no.	Day 1	Day 2	Day 3	Day 4	Day 5
1	Quat. ammonium 200 p.p.m. 760	Chlorine 400 p.p.m. 740	Chlorine 200 p.p.m. 610	Quat. ammonium 400 p.p.m. 1,300	Water 1,700
2	Chlorine 200 p.p.m. 1,100	Quat. ammonium 400 p.p.m. 1,000	Chlorine 400 p.p.m. 9,800 ^b	Water 1,000	Quat. ammonium 200 p.p.m. 70,000 ^b
3	Water 540	Chlorine 200 p.p.m. 740	Quat. ammonium 200 p.p.m. 1,900	Chlorine 400 p.p.m. 1,500	Quat. ammonium 400 p.p.m. 650
4	Chlorine 400 p.p.m. 1,700	Water 1,100	Quat. ammonium 400 p.p.m. 510	Quat. ammonium 200 p.p.m. 1,600	Chlorine 200 p.p.m. 840
5	Quat. ammonium 400 p.p.m. 1,900	Quat. ammonium 200 p.p.m. 2,000	Water 92,000 ^c	Chlorine 200 p.p.m. 60,000 ^c	Chlorine 400 p.p.m. 1,100

^a Expressed as bacteria per ml.

^b Mastitis.

^c Teat cup touched bedding.

Trials II, III, IV and V were run with no further occurrence of contamination. Data for the five trials are shown in table 2. The mean figures shown for Trial I exclude the four instances when the count was high due to uncontrolled influences.

TABLE 2
The effect of hypochlorite and quaternary ammonium solutions, when used in udder washes, upon the plate count of milks^a

Trial no.	Chlorine 200 p.p.m.	Chlorine 400 p.p.m.	Quarternary ammonium 200 p.p.m.	Quarternary ammonium 400 p.p.m.	Water
I	823 ^b	1,260 ^b	1,565 ^b	1,072	1,085 ^b
II	2,208	2,370	2,812	2,756	1,946
III	1,438	1,372	930	1,448	1,528
IV	2,461	1,109	2,176	1,198	2,174
V	5,821	2,170	2,234	1,180	1,390
Mean	2,550	1,656	1,943	1,531	1,625

^a Mean of 5 observations on each treatment expressed as bacteria per ml.

^b Mean of 4 observations.

An analysis of variance was run on Trials II, III and IV, which represent the trials on evening milkings with no data missing. No significant difference in treatments could be observed. There was a highly significant difference between cows.

An analysis then was run on all five trials, using the missing data technique (7) for Trial I. Again there was no statistically significant difference between treatments. The variations due to cows were highly significant.

A comparison of the evening and morning counts between Trials IV and V was made. The mean count for the 25 morning observations was 2,559 bacteria per ml.; for the corresponding evening counts the mean was 1,824. This difference was not statistically significant.

SUMMARY AND CONCLUSIONS

The effect of various udder washes upon the plate count of milk was studied using two concentrations of chlorine, two concentrations of quaternary ammonium, and clean water. Milking was done with previously autoclaved milking machines and the raw milk plated on tryptone-glucose-extract-milk agar before growth could take place. No differences in count could be attributed to treatments. A large degree of variation was observed between individual cows. On 25 pairs of observations, no significant difference in counts could be observed between night and morning milkings.

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THE NUTRITION OF THE NEWBORN DAIRY CALF. I. CHANGES IN THE TRYPTOPHAN CONTENT OF THE BLOOD PLASMA FOLLOWING BIRTH AND THE INGESTION OF COLOSTRUM

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Recent work at the University of Illinois (6) has shown that calves do not require a dietary source of nicotinic acid when fed a nicotinic acid-free synthetic milk. These calves were placed on a synthetic milk diet after receiving colostrum for 48 hours and were continued on the experimental diet for 12 weeks. A study of the urinary excretion of nicotinic acid and its metabolic products, nicotinamide, nicotinuric acid and N¹-methylnicotinamide, showed that the total excretion dropped rapidly following colostrum feeding, and then remained at a fairly constant level throughout the remainder of the experimental period.

Contemporary work (7, 9, 10) has provided evidence that tryptophan is the precursor used in the *in vivo* synthesis of nicotinic acid. The relatively high blood plasma nicotinic acid values observed following a 48-hour colostrum-feeding period by the Illinois workers (6), plus the fact that other workers (8) have reported rather low nicotinic acid values for cows' colostrum, suggest the desirability of studying the tryptophan content of colostrum and the changes which occur in plasma concentration following colostrum feeding.

A review of the literature revealed no information on the concentration of tryptophan in colostrum or in newborn calf blood plasma. However, it is known that the proteins of milk are relatively well supplied with this essential amino acid. Data in the literature show 0.31 to 0.32 per cent of tryptophan in whole milk powder (3), from 1.09 to 1.31 per cent of tryptophan in casein (2, 3, 4, 5, 11), and from 1.74 to 2.66 per cent tryptophan in lactalbumin (2, 3, 4, 5). The nature and concentration of proteins in cows' colostrum suggest that it should supply an abundance of tryptophan for the nutrition of the newborn calf.

EXPERIMENTAL PROCEDURE

Tryptophan determinations were made on the first- and second-milking colostrum from 10 cows and on the blood plasma of 13 calves at the time

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¹ A graduate student at The Ohio State University supported by the Government of India.

of birth and on the third, seventh, and fourteenth day following birth. Determinations also were made on the blood plasma of 10 of these calves on the twenty-first day following birth. These calves all were born in the Ohio State University Dairy Herd during August and September, 1947. Tryptophan determinations also were made on the whole milk and blood plasma of 15 cows for comparison. Samples of the colostrum as well as of the milk were taken from the complete milking.

The *p*-dimethylaminobenzaldehyde method of Bates (1) as modified by Graham *et al.* (2) was employed for tryptophan estimation. An Evelyn photoelectric colorimeter with a 550-m μ filter was used. The *K* value was obtained by carrying out the procedure with known quantities of *L*-tryptophan² and checked with known quantities of *D*-*L*-tryptophan.³

RESULTS

The analytical data obtained are presented in the tables. It will be noted that the tryptophan level in blood plasma is quite low in the newborn calf, averaging 0.46 mg. per g., wet basis (table 1). In every instance there

TABLE 1

Changes in the blood plasma tryptophan of calves following birth

Calf no. ^a	Blood plasma tryptophan				
	Age in days				
	0 ^b	3	7	14	21
	(mg./g.) ^c				
42S	0.46	1.05	1.10	1.04	0.86
41S	0.46	0.99	1.05	0.84	0.75
503H	0.47	1.16	1.16	0.99	0.82
43S	0.43	0.55	0.65	0.62
355A	0.56	1.28	1.01	0.92	0.96
357A	0.75	1.43	1.53	1.43	1.12
329G	0.41	0.71	0.74	0.66	0.64
330G	0.41	0.81	0.84	0.91	0.55
331G	0.45	0.87	0.82	0.70	0.81
332G	0.44	0.95	0.77	0.47	0.89
272J	0.40	0.98	0.86	0.76	0.76
373J	0.48	0.70	0.91	0.76
507H	0.34	0.81	1.14	1.09
Av.	0.46	0.94	0.96	0.86	0.81

^a The letter following the number designates breed: S = Brown Swiss, H = Holstein, A = Ayrshire, G = Guernsey, J = Jersey.

^b These samples all were taken before the first feeding of colostrum.

^c Wet basis.

was a marked increase during the first 3 days, and the average on the third day was approximately double that of the newborn calf. The highest aver-

² Supplied through the courtesy of Dr. F. E. Deatherage, Department of Agricultural Chemistry.

³ Supplied through the courtesy of Merck and Co., Rahway, New Jersey.

age level was observed on the seventh day, following which there was a slight decline. At no time during the first 21 days was a level attained which was as high as that of the adult cow, although in a few instances on the third, seventh, and fourteenth days values were recorded which were within the range found for adult cows, as shown in table 2.

TABLE 2
Tryptophan content of blood plasma and milk of dairy cows

Cow no. ^a	Lactation no.	Days in milk	Blood plasma tryptophan (mg./g.) ^b	Milk tryptophan (mg./g.) ^b
191A	10	44	1.54	0.78
214A	10	146	1.80	0.82
269A	4	35	1.38	0.62
410H	3	75	1.54	0.71
433H	2	218	1.20	0.73
460H	1	13	1.20	0.68
277G	3	28	1.02	0.70
221G	6	250	1.55	0.84
275G	2	16	1.39	0.78
19S	1	69	1.20	0.69
11S	3	236	1.53	0.81
7S	9	39	1.51	0.75
335J	1	17	1.12	0.82
337J	1	48	1.60	0.75
325J	2	48	1.20	0.87
Av.			1.38	0.75

^a The letter following the number designates breed: A = Ayrshire, H = Holstein, G = Guernsey, S = Brown Swiss, J = Jersey.

^b Wet basis.

Table 3 shows colostrum to be a rich source of tryptophan. On a wet basis, first-milking colostrum contains about five times as much as normal milk. Second-milking colostrum is about three times as high in tryptophan as normal milk. One pound of average first-milking colostrum would provide approximately 1.74 g. of tryptophan, while a pound of normal milk would supply only about 0.34 g.

The tryptophan content of a 20-lb. lot of fat-free moisture-free colostrum which had been obtained from the first and second milkings of several cows was compared with that of samples of spray-dried and drum-dried commercial nonfat-dry-milk solids. Analyses of these materials gave results of 13.5, 7.9, and 7.7 mg. of tryptophan per g. for the dry colostrum, the spray-dried powder, and the drum-dried powder, respectively. These results are in agreement with what one might expect, since a greater proportion of the nonfat solids of colostrum is protein and a greater proportion of the protein of colostrum consists of fractions which are somewhat higher in tryptophan than casein.

These results suggest that the high excretion rate of nicotinic acid and its metabolic products found by the Illinois workers (6) may have resulted

from the high tryptophan consumption during the first 48 hours. It seems logical to assume that nicotinic acid synthesis in the very young calf must be dependent upon some dietary precursor since microbiological synthesis

TABLE 3
Tryptophan content of colostrum

Cow no. ^a	Tryptophan	
	1st milking	2nd milking
	(mg./g.) ^b	(mg./g.) ^b
373A	4.61	3.15
265A	4.60	2.42
191A	4.62	3.26
269A	5.94	3.93
273H	4.32	3.55
464H	2.82	2.30
460H	4.90	3.16
337J	1.42	1.05
281G	3.15	1.75
277G	2.16	1.20
Av.	3.85	2.57

^a The letter following the number designates breed: A = Ayrshire, H = Holstein, J = Jersey, G = Guernsey.

^b Wet basis.

in the rumen is a doubtful source of this nutrient at so early an age. On the other hand, it may be that a relationship exists between tryptophan and nicotinic acid similar to that known to occur between methionine and choline.

A study of the effects of colostrum consumption on the excretion of nicotinic acid and its precursors is being contemplated.

SUMMARY

A study of the tryptophan content of the blood plasma of calves showed average values of 0.46, 0.94, 0.96, 0.86, and 0.81 mg. per g. wet basis for the newborn calf and at 3, 7, 14 and 21 days of age, respectively.

The amount of tryptophan in first-milking colostrum was found to be about five times as high and that of the second milking about three times as high as that of normal milk.

These results are discussed in the light of tryptophan being the possible precursor for the *in vivo* synthesis of nicotinic acid.

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A COMPARISON OF VACUUM AND STEAM DISTILLATION FOR DETERMINING THE VOLATILE ACIDITY OF EVAPORATED MILK

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The acidity and changes in acidity of milk are known to be of importance in milk processing and storage. Many workers have shown that heat causes the formation of acids in milk. As early as 1895 Cazeneuve and Haddon (2) reported that the acid formed by heating was mainly formic acid. This view is in accordance with the recent work of Gould (7) and Gould and Frantz (8). Since most workers report the chief volatile acid produced by heating milk to be formic, the importance of other volatile acids appears questionable.

The use of steam distillation for removing volatile acids from sterilized evaporated milk may or may not be the best procedure. Destruction of milk constituents during steam distillation is indicated by a marked browning of the milk and liberation of volatile sulfides (at proper pH). Although reports show that the steam distillation of raw skim milk or whole milk resulted in the formation of no appreciable amount of formic acid, (7, 8) this does not show what steam distillation may do to heated milk. Also, volatile acids other than formic possibly may be affected. For instance, glycerides of lower fatty acids may be split during the steam distillation of milk.

The use of vacuum distillation in removing volatile constituents from foods has been suggested by Fischbach (5) and has been used successfully by other workers. Vacuum distillation seemed to be a logical method for removing the volatile acids from milk without the use of excessive heating. This investigation was concerned with a comparison of vacuum distillation and steam distillation as a means of studying formic and total volatile acids in heated milk.

EXPERIMENTAL PROCEDURE

Vacuum distillation. The distilling setup consisted of pryex ground-joint equipment. Essential pieces were a 3-l. round-bottom distillation flask, a 300-mm. spiral (Graham) condenser, a 1-l. receiving flask, and suitable connecting tubes and adapters. Constant pressure was maintained by means of a Cartesian diver-type manostat (6).

In the vacuum method finally adopted 500 ml. of reconstituted milk (1 part evaporated milk to 1.2 parts water) was acidified to pH 1.5 (approximately) by running in 100 ml. of *N* sulfuric acid while the milk

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was being agitated with a mechanical stirrer. The acidified milk was distilled at a pressure of 24 mm. mercury and at a rate of approximately 400 ml./hr. The boiling temperature was 24–25° C. The volume of the distilland was maintained constant by running boiled distilled water, from a calibrated flask protected by a soda-lime tube, into the distilling flask through a glass tube and stopcock arrangement, and checking the volume of water run in against the volume of distillate.

For the determination of total volatile acids, 600 ml. of distillate was collected and titrated under nitrogen to phenolphthalein with 0.1 *N* sodium hydroxide. A blank correction of 0.09 ml. of 0.1 *N* sodium hydroxide for the amount of alkali required to change the indicator in 600 ml. of boiled distilled water was deducted from the titration. Total volatile acids in the distillate were calculated as formic acid.

For the determination of formic acid the entire distillate then was concentrated to about 100 ml. by boiling with 200 ml. of 1 per cent barium carbonate suspension. The barium carbonate was filtered off and the formic acid determined by the A.O.A.C. gravimetric procedure (1).

Steam distillation. The apparatus for steam distillation was essentially the same as that used for vacuum distillation except for the necessary provision for a steam generator and a soda-lime tube connected to the receiving flask outlet in place of vacuum equipment.

The distillation procedure was similar to the vacuum distillation except for the use of steam at atmospheric pressure and the acidification of the diluted evaporated milk sample. The diluted sample was acidified in this case with only 80 ml. of *N* sulfuric acid and 20 ml. of water to lower the pH to approximately 2.0 (as against pH 1.5 and a much lower temperature with the vacuum method).

The steam distillation procedure differed from that used by Gould and Frantz (8) and Gould *et al.* (9) in the following details: (a) In the present work the size of the sample was doubled. (b) Volatile acids were collected in the distillate rather than in a barium carbonate trap. (c) The ratio of distillate to distilland was 1:1 instead of 3.33:1.

Total volatile acids and formic acid in the steam distillate were determined by the procedure employed with the vacuum distillate.

RESULTS

Distillation curves. Calculation of formic acid and total volatile acids in the milk was based on distillation curves of formic acid determined in the particular apparatus used. The curves presented in figure 1 were plotted from data obtained by distilling dilute aqueous solutions of pure formic, *n*-butyric, and caproic acids under the same conditions and in the same apparatus used in distilling milk samples. The method of plotting on inverted semi-log paper was copied after Dyer (4). Actually, the log of

the percentage of volatile acid remaining in the distilland was plotted against total volume of distillate collected, giving a straight line. However, since the main purpose of the figure is to show the per cent of acid recovered in the distillate, the data were plotted on inverted semi-log paper.

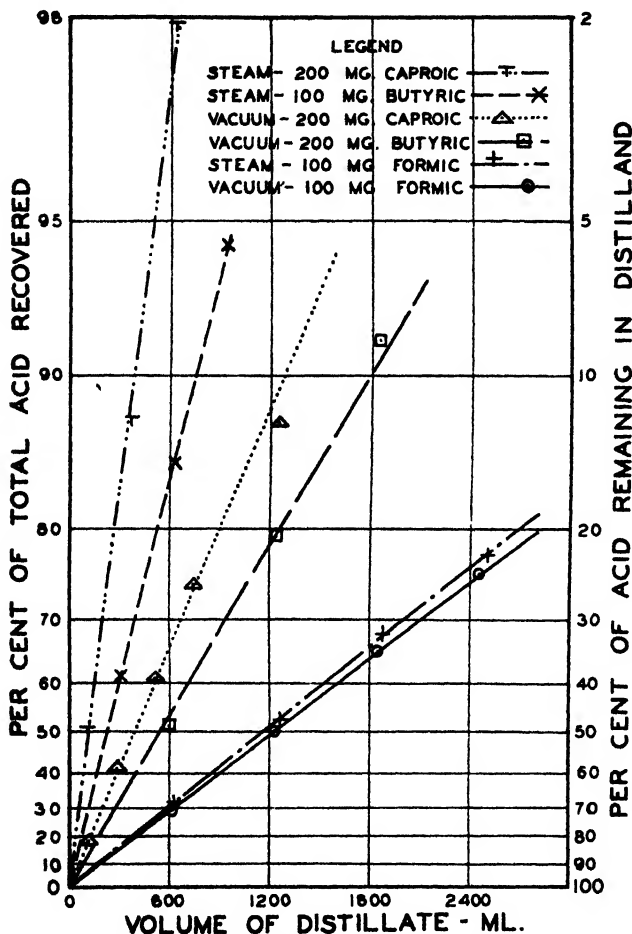


Fig. 1. The rate of vacuum and steam distillation of various acids from aqueous solutions under experimental conditions similar to those used with milk.

The rates of distillation of formic acid with steam and vacuum were about the same, whereas *n*-butyric acid and caproic acid were distilled much more rapidly with steam. Assuming that the rate of distillation of formic acid from the diluted, acidified milk samples was the same as the rate from acidified water, the per cent of the total formic acid in any given volume

of distillate could be determined from the vacuum or steam distillation curve, and thus the amount of formic acid originally present in the sample could be calculated. For example, 600 ml. of distillate (by the vacuum method) would contain 28.7 per cent of the total formic acid in the sample.

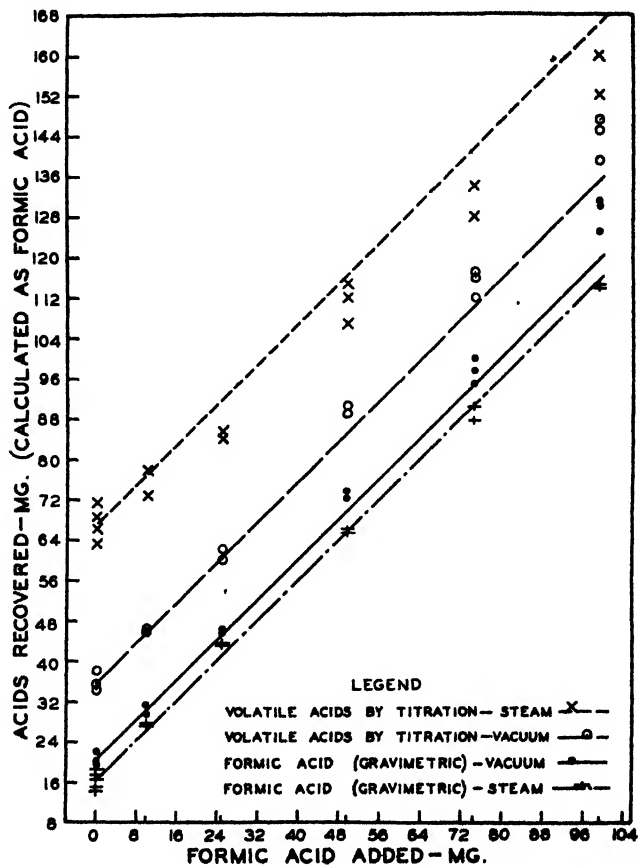


FIG. 2. The recovery of formic acid added to 500-ml. samples of reconstituted sterilized evaporated milk as determined by vacuum and steam distillations.

If the distillate was found to contain 10 mg. of formic acid, the total formic acid in the sample would be 34.8 mg.

In the case of the total volatile acidity determinations, an arbitrary figure was calculated. From the total volatile acidity (as formic acid) in the distillate, the amount in the sample was estimated on the basis of the formic acid distillation curve. This gave values which undoubtedly are high but which permitted a rough comparison of total volatile acidity,

formic acid values, and, by difference, the volatile acids not formic in the distillates.

Recovery of formic acid added to sterilized evaporated milk. For recovery experiments, 48 cans of fresh commercial sterilized evaporated milk were placed in a refrigerator until used. Determinations of volatile acidity and formic acid at intervals during the course of the recovery experiments showed no detectable increase in these constituents up to the time the work was completed.

Amounts of solution containing from 10 to 100 mg. of formic acid were added to diluted evaporated milk samples by running the solution through a capillary into milk which was being agitated with a mechanical stirrer. Approximately 600 ml. of distillate from the 600 ml. of diluted acidified sample was collected by vacuum or steam distillation. Titrations of total volatile acids and gravimetric determinations of formic acid were made on the distillates. Values were corrected to 100 per cent recovery on the basis of the vacuum or steam distillation curve for formic acid.

Figure 2 is a graphic representation of data obtained in determinations of total volatile acid and gravimetric formic acid by the steam and vacuum distillation methods. The four lines were drawn through points obtained by taking the sum of the average acid value obtained on the control samples by each method and the amount of formic acid added. Thus, these lines represent values which *should have been obtained* if the recovery of the added formic acid were 100 per cent in all cases. The points plotted show actual values obtained on individual samples containing from 0 to 100 mg. of added formic acid.

- The differences in base values obtained on control samples are clearly shown here. Total volatile acidity as determined by the steam distillations was almost double that determined by the vacuum method. The base value for formic acid determined gravimetrically was higher by 3.9 mg. with the vacuum method than with the steam procedure.

With the vacuum distillation procedure, values for total volatile acidity and formic acid in the control group of samples were quite consistent considering the small quantities present in this milk. The results indicate that the method of calculating the amount of formic acid in the milk from the comparatively small amount recovered in the distillate was at least fairly accurate. The recovery of formic acid calculated on the basis of the titration of the distillate agreed well, in most cases, with the figure calculated on the basis of the gravimetric formic acid determination. Recoveries were slightly high, but generally within the range of accuracy to be expected in work of this kind.

With the steam distillation procedure, recoveries of added formic acid by the gravimetric method were very satisfactory in amounts of 50 mg. or more. With smaller amounts of formic acid the recoveries tended to run high. A

point of particular interest here was the erratic and generally low recovery of formic acid as calculated from the titrations of the steam distillate, with recoveries from 58.7 to 105.7 per cent, the average being 83.8 per cent.

The low recovery of formic acid by titration of the steam distillates apparently was due to the fact that values for volatile acids not formic were lower in distillates from samples to which formic acid had been added, since gravimetric determinations of formic acid in these same distillates showed no loss. The vacuum procedure did not give this difference between titration and gravimetric values. As shown in figure 3, there seemed to be a definite relationship between the amount of formic acid added to the milk

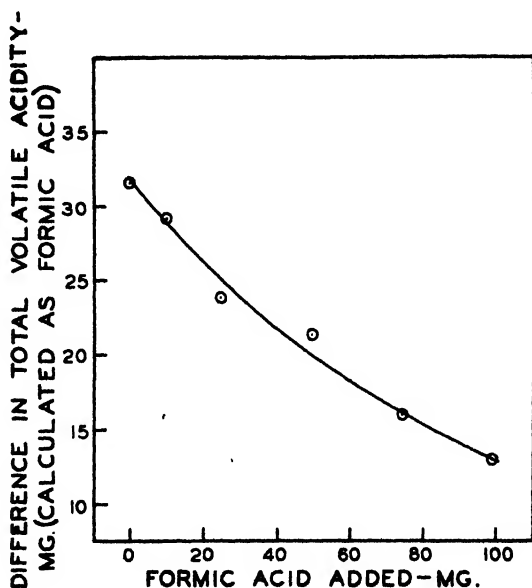


FIG. 3. The effect of amount of added formic acid on the difference between steam and vacuum distillation results for total volatile acidity.

and the difference in volatile acidity values between steam and vacuum distilled samples; i.e., as the amount of added formic acid was increased, the difference in volatile acidity between steam and vacuum distilled samples decreased. Consideration of volatile acids not formic may give an explanation for these results.

Volatile acids not formic. Calculation of volatile acids not formic was made on steam and vacuum distilled samples by subtracting the gravimetric formic acid values from the total volatile acidity obtained by titration. In figure 4, volatile acids not formic were plotted against added formic acid. This demonstrated that: (a) Values for volatile acids not formic were much

higher by the steam distillation. (b) The steam distillation showed a definite drop in volatile acids not formic when formic acid was added to the milk. (c) The vacuum distillation values for volatile acids not formic remained fairly constant when the formic acid content of the milk was increased.

Possible explanations of the higher volatile acidity values obtained with steam distillation may be: (a) evolution of carbon dioxide during the steam

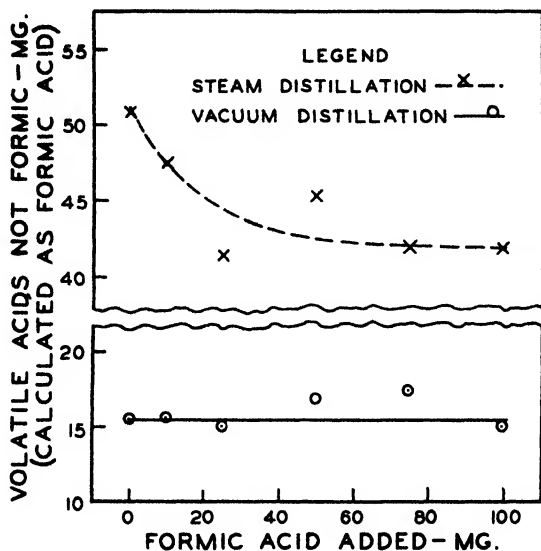


FIG. 4. The relation of amount of added formic acid to the volatile acids not formic as determined by vacuum and steam distillation.

distillation, (b) hydrolysis of glycerides of the lower fatty acids, and (c) more rapid distillation of butyric and higher volatile acids with steam than with vacuum.

The effect of boiling the steam and vacuum distillates to eliminate carbon dioxide is shown in table 1. In these experiments the distillate was titrated with sodium hydroxide under nitrogen as usual. Then standard hydrochloric acid was added in amount equivalent to the sodium hydroxide used in titration. The distillate was heated to boiling as rapidly as possible, boiled for 30 seconds, cooled under protection of a soda-lime tube, and titrated again with sodium hydroxide.

Boiling resulted in considerable loss of acidity in the steam distillate but slight loss of acidity in the vacuum distillate. The amount of formic acid, as determined by the gravimetric procedure, was not decreased appreciably by boiling either steam or vacuum distillate. Negative tests for sulfides on

the steam distillate eliminated the possibility that hydrogen sulfide was causing the high titrations. By placing barium hydroxide solution in the receiving flask during the steam distillation, it was found that carbon dioxide was evolved even after 2 hours of distillation and aspiration with carbon dioxide-free nitrogen. This supports the hypothesis that a part of the difference between volatile acidity values as determined by vacuum and steam distillation was due to dissolved carbon dioxide in the steam distillate. Since a marked browning of the milk occurs during steam distillation, the evolu-

TABLE 1

Effect of boiling evaporated milk distillate on total volatile acidity titration

Distillation procedure	HCOOH added	0.1 N NaOH required for approximately 600 ml. of distillate		
		Unboiled	Boiled	Difference
	(mg.)	(ml.)	(ml.)	(ml.)
Steam	0	4.23	3.22	1.01
	24.8	5.64	4.22	1.42
	49.6	7.63	6.04	1.59
Vacuum	0.0	2.19	2.18	0.01
	9.9	2.86	2.66	0.20
	49.6	5.57	5.44	0.13
	74.4	7.15	6.91	0.24

tion of carbon dioxide during this treatment would be expected. Tarassuk (10) and Coulter (3) have observed carbon dioxide evolution during browning of milk by heat. These reactions are reported to be accelerated by oxygen (10) and inhibited by reducing agents such as formaldehyde and sodium bisulphite (11).

Even after making correction for the loss of acidity on boiling of the steam distillate, its acidity would be higher by about 34 per cent than that of the vacuum distillate on samples to which no formic acid was added, indicating that other factors contributed to the difference in volatile acidity values obtained by the two methods.

Steam distillation of 5 g. of pure tributyrin homogenized with water and distilled under conditions used on milk was tried and resulted in hydrolysis of 3.5 per cent of the tributyrin in 1.5 hours. Vacuum distillation of tributyrin in water did not hydrolyze any of the glyceride. This indicated the possibility of splitting glycerides of lower fatty acids during the steam distillation of milk. However, mixed glycerides such as occur in milk fat probably would be more stable than tributyrin, and so the error due to hydrolysis of glycerides during the steam distillation may or may not be great.

From observations of the distillation curves (Fig. 1) it is evident that the more rapid distillation of butyric and caproic acids with steam could account

for at least part of the difference in volatile acidity as determined by vacuum and steam distillation in these experiments, since total volatile acids in the sample were calculated on the basis of the distillation rate curves of formic acid. However, the amounts of individual acids of higher molecular weight than formic in the distillates were not known, and it is impossible to determine from data obtained just how much this error in calculating total volatile acids contributed to the difference in values obtained by steam and vacuum distillation.

The available data do not show the specific cause for the decrease in volatile acids not formic obtained by steam distillation when formic acid was added to the milk. The limited results obtained by titrations before and after boiling of steam distillates do not indicate that this decrease was due to inhibition of evolution of carbon dioxide by the added formic acid. There seems to be a possibility that formation and/or distillation of part of the volatile acids not formic was inhibited by the presence of formic acid.

SUMMARY

1. A method of determining formic acid and volatile acid values in heated milk by means of vacuum distillation at constant volume is presented. Results of experiments to determine the recovery of added formic acid from sterilized evaporated milk by the steam and vacuum distillation procedures are discussed.

2. The formic acid content of reconstituted evaporated milk averaged about 32 mg./l. by steam distillation and about 40 mg./l. by vacuum distillation.

3. The total volatile acid content of reconstituted evaporated milk, expressed as formic acid, averaged 134 mg./l. by steam distillation and 70 mg./l. by vacuum distillation.

4. The vacuum and steam distillation procedures both gave satisfactory recovery of added formic acid from sterilized evaporated milk by the gravimetric method.

5. There was no evidence of production of formic acid during steam distillation as far as the accuracy of the methods could detect.

6. Titrations of volatile acids on the steam distillates were considerably higher than on the vacuum distillates. The difference could be due to continuous evolution of carbon dioxide from the milk during steam distillation, the more rapid distillation rate of butyric and caproic acids with steam, and/or splitting of glycerides of fatty acids during steam distillation.

7. The addition of formic acid to the milk appeared to reduce the amount of volatile acids not formic obtained by steam distillation.

8. The vacuum distillation showed an advantage over steam distillation where a consideration of the total volatile acids in the distillate was desired.

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CURD TENSION TEST AND CURD NUMBER TEST APPLIED TO MARKET HOMOGENIZED MILK IN PHILADELPHIA— DEFINITION OF A SOFT CURD MILK

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Since the curd number test was described in 1942 (3), this method as well as the curd tension test has been applied regularly in this laboratory to homogenized milks marketed in Philadelphia. The present paper gives a survey of the results and discusses the relationship between curd tension, which deals with the toughness of the curd when milk coagulates, and the curd number, which deals with the size of the curds formed.

The term "soft curd milk" is entirely of American origin and until recently has been practically unknown in other countries. Hill (2) was the first to call attention to the variance in the curd-forming properties of milk, and he designed a special apparatus, manipulated by hand, for testing the toughness of the curd formed under standard conditions. Hill selected a temperature of 95° F. (35° C.) and prescribed a special coagulation solution.

The curd test later was subjected, by a specially appointed committee, to a critical study which resulted in detailed directions for the performance of the test (1). The Hill curd tension meter has been replaced by a mechanically operated apparatus designed by Chambers and now generally used. The curd number test (3) was developed in this laboratory to study closely another aspect of curd formation, namely, the size of the curd. In connection with this study, a baby feeding study was carried out (4).

It generally is agreed that a low curd tension in milk is desirable, especially for infants and people with weakened digestion. A low curd tension is indicative of a soft curd, but even more important is the formation of small curds that will yield a large surface for the action of the digestive juices.

The main objective of this study has been to investigate the relationship between curd tension and the formation of curds as measured by the curd number test, and especially to determine at which curd tension a formation of generally small curds is assured in homogenized milk.

EXPERIMENTAL

A period of one year, June, 1946, to June, 1947, was selected to include all possible seasonal variations. In all, 208 curd number tests, as well as curd tension tests, were performed on market homogenized milks.

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The results of all curd tension values over 10 g. and their corresponding curd numbers are recorded in table 1. Curd tension values below 10 g. frequently were found, indicating extremely soft curd milks. These samples were regarded as without significance for this study, in which the upper

TABLE 1
The relationship of curd tension to curd number
(June, 1946-June, 1947)

No. of samples	Curd tension	Corresponding curd nos.		
		Max.	Min.	Av.
22	10	228	201	214
20	11	229	200	211
18	12	236	200	210
17	13	229	195	210
16	14	231	195	207
23	15	216	195	205
10	16	221	178	202
5	17	214	167	185

limit value for curd tension and the corresponding lower limit value for curd number, characterizing soft curd milks, were sought. Table 1 shows curd tensions from 10 to 17 g. and corresponding curd numbers. Briefly, the curd numbers are arrived at by adding the per centum weight of the curds, divided into three different sizes, large, medium, and small, after having multiplied these figures by one, two, and three, respectively. The presented figures total 131 in number, which means that the rest of the curd tensions, 77 or 37 per cent, were below 10 g. The variance in curd numbers from 10 to 16 g. is not great. None of the figures except one of 178, corresponding to curd tension 16, was below 195. Only five curd tensions of 17 g. were recorded and none over this amount; of these five, the three

TABLE 2
The curd numbers of samples with high curd tensions (17-29)

No. of samples	Curd tension	Corresponding curd nos.		
		Max.	Min.	Av.
8	17	214	135	185
4	20	191	171	178
4	21	180	143	166
3	22	184	164	174
3	28	156	131	139
2	29	171	149	160

relatively low curd numbers, 167, 169 and 175, respectively, indicate the downward trend when the curd tensions increase beyond 16.

From the records taken before 1946, all curd numbers corresponding to a curd tension of 17 g. are recorded in table 2. Of these eight figures, only

two are over 200. The average is the same (185) as for the one-year period tabulated in table 1. Over a period of about 6 years, a few curd tensions of 20 g. and over were encountered. These are tabulated in table 2. Because of their extreme scarcity, they do not have any significance whatsoever in the total picture of curd tensions and curd numbers but are interesting because they show the downward trend in curd numbers with increasing curd tensions. Of special interest are the milks with curd tensions of 20 g., as this is the upper limit value for a milk labeled as "soft curd" according to rules in some states. The average of the four recorded curd tensions of 20 g. is 178, and only one is close to 200. It may seem a little strange that the average curd numbers for curd tensions 22 g. and 29 g. are higher than the average curd numbers for curd tensions 21 g. and 28 g., respectively, but this is only because of the relatively few tests involved. With more tests the higher curd tensions undoubtedly would give corresponding lower average curd numbers. This demonstrates, however, that milks with closely similar curd tensions often vary widely in their curd numbers.

DISCUSSION

In the curd number test study (3) the milk curds were divided into three sizes: large curds, caught by a sieve with 0.5-inch mesh; medium-size curds, caught by a sieve with 0.10-inch mesh; and small curds, caught by a sieve with 0.01-inch mesh.

Breast milk usually has curds only of the small size and accordingly will get the highest curd number rating of 300. In the baby feeding study connected with the curd formation study (4), the great majority of milks fed to the babies had curd numbers of 200 and above (1). This milk was well tolerated, and it was suggested at that time that a milk with curd number 200 and over should be labeled as a soft curd milk.

Hundreds of curd specimens have been studied in an effort to determine what sort of curd combinations represent a curd number of about 200 (195-205). Theoretically it is possible to reach this curd number in many different ways. Even a mix of 50 per cent large curds and 50 per cent small curds with no curds of the middle size would give a curd number of 200. Of course, this combination would not be desirable and such a case never has been encountered. Usually a considerable number of the middle-size curds are present, and for a curd number around 200, the large curds will range from 5 to 15 per cent of the total curd weight. The curd numbers of homogenized milks usually are considerably above 200 and consequently show no large curds at all. Furthermore, it is a matter of experience that the large curds for a curd number of 200 are relatively small or close to the mesh size of 0.5-inch in diameter.

Since milk with a curd number of 200 and above, corresponding to a curd tension of 15 g. or less, is well tolerated by infants in general (4),

and since milks with curd tensions above 15 g. contain an increasing number of specimens with curd numbers below 200 as the curd tension increases, it seems reasonable to label milks with curd tensions of 15 g. or below as "soft curd" milks, meaning both "soft" and "small curd" milks.

SUMMARY

The curd tensions and corresponding curd numbers (curd sizes) of market homogenized milk in Philadelphia have been recorded over the period of one year. On the basis of this material, combined with experiences from baby feeding studies and more than 5 years of constant testing of curd tensions and curd numbers, it is suggested that a "soft curd" milk be defined as a milk with a curd tension of 15 g. or below. Since this corresponds to a curd number of 200 or above, it indicates a milk with both soft and small curds.

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VARIATIONS IN YIELD OF MILK UNDER THE PENKEEPING SYSTEM IN BRAZIL

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The program for dairy cattle improvement in the state of Minas Gerais in Brazil includes investigating the effects of the special conditions existing under the penkeeping system (*sistema de retiros*) of dairy cattle management. This system is widely used in the *Zona da Mata* (originally a forested region) in the southeastern part of Minas Gerais. The penkeeping system and some of the problems encountered already have been described by Rhoad (5) and by Carneiro (2). Some of the findings from these studies appear to be of general interest.

In the penkeeping system the cattle are kept on pasture the year around. They are divided into *retiros* or pens of 20 to 40 head each. The cows are with their calves in the pasture during the day but are penned up in the evening and remain separated from the calves until they are milked in the morning. As a rule the only feed they get is pasture, but in recent years some use has been made of chopped sugar cane, grass silage, and even cottonseed meal and wheat bran during the dry season. The climate is hot and the annual rainfall is high, with two definite seasons, a rainy one from October to March and a dry season from April to September. The most important among the pests and diseases include ticks, "*berne*" (*Dermatobia hominis*), flies, worms, foot-and-mouth disease, anthrax, blackleg, pneumonia, and tick fever.

The principal objectives of the present study were to determine: (a) How much the average milk production changed from one year to another and the extent to which these year-to-year changes were part of a general time trend or were only irregular variations. (b) How the milk production varied at different times of the year. (c) The shape of the lactation curve with advancing lactation. The findings concerning the sex ratio and preliminary observations on heritability also are reported.

EXPERIMENTAL PROCEDURE

The records came from "Niagara" farm near Leopoldina, Brazil. This is a private farm which kept grade Simmenthaler cattle under management

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practices thought to be typical of the penkeeping system in that region. The records were for lactations begun in the years 1930 to 1937. The number of cows in milk ranged from 233 to 318. Milk yields were measured on the first and the sixteenth of each month. All the data on yield are in liters. Under the penkeeping system either the death of the calf or sickness of the cow affects production sharply. Lactations were considered abnormal and were omitted from this analysis if the cow was seriously sick, as from foot-and-mouth disease, or if the calf died or the cow was sold early in the lactation. Of the 2,177 lactations recorded, only 1,318 (slightly over 60 per cent) were considered normal. Table 1 shows for each year the number of lactations and the means and standard deviations for length of lactation and for milk yield in the normal lactations. The ratio of normal to abnormal lactations fluctuated widely from year to year, as might be expected from the fact that outbreaks of foot-and-mouth or other disease often would cause a heavy loss in some *retiros* or in some years but not in others.

TABLE 1
Normal, abnormal and total lactations by years

Year	No. of lactations			Milk yield (l.)		Lactation period (days)	
	Normal	Abnormal	Total	Mean	σ	Mean	σ
1930	194	70	264	1,105	386	289	66
1931	97	136	233	1,144	402	316	82
1932	161	81	242	1,282	344	313	67
1933	75	195	270	1,129	348	287	72
1934	196	56	252	1,207	365	305	64
1935	213	70	283	1,261	413	301	65
1936	187	128	315	1,226	407	285	63
1937	195	123	318	1,242	413	304	61
Summary	1,318	859	2,177	1,209	393	299	67
				± 11		± 2	

RESULTS

Year-to-year differences in yield and in length of lactation period. Table 1 shows how the means varied from one year to the next. Table 2 shows the analysis of variance between and within years. Since the effect of year on length of lactation only bordered on statistical significance and was slight in any case, its analysis was carried no further.

The mean yield differed from year to year with unmistakable statistical significance. The analysis to test whether those year-to-year differences were wholly irregular or whether a part of them could be attributed to a straight-line trend also is shown in table 2. The trend is an average increase of 16.7 l. per year, ($Y = 1147.4 + 16.7X$) but the yearly means changed too irregularly for statistical significance to be assured. Other

things besides the steady trend (if that actually is real) obviously have much to do with causing the mean to be high in some years and low in others. A rough computation of the variance components indicates that about 40 per cent of the variance caused by year-to-year changes in the mean can be at-

TABLE 2
Variation in milk yield between and within years

Source of variation	<i>d/f</i>	Milk yield		Length of lactation	
		Mean square	<i>F</i>	Mean square	<i>F</i>
Total	1,317	154,284		4,423	
Regression	1	1,924,583	4.16	11,099	2.53*
Yearly means from the regression	6	462,486	3.05**		
Between cows within years	1,310	151,521		4,388	

* = Significant.

** = Highly significant.

tributed to the straight-line trend. The other 60 per cent of the year-to-year variance comes from causes which had irregular incidence from one year to the next. However, the year-to-year variations were not a large part of the total causes of individual variation, since the variance component for year-to-year differences, including the trend, is only about 2 per cent of the variance between records made within the same year. The individual variation found between records made within the same year (necessarily by different cows) is so large that even an indicated increase of nearly 1.4 per cent per year is by comparison a small source of variation. This small increase, however, eventually would mean much to the dairy industry if it is real and if it continues for many years.

Season of year. Only a portion of the data was used for measuring how yield varied with the season of the year, 50 of the normal lactations being

TABLE 3
Variance of daily milk yields between and within months of the year

Source of variation	<i>d/f</i>	Mean square	<i>F</i>
Total	3,876	2.722	
Between months	11	29.236	11.05**
Within months	3,865	2.646	

** = Highly significant.

selected from each of the 8 years. The selections were random except that some effort was made to get the calving dates equally distributed among the 12 months, so that stage of lactation would not be confounded with season of year. This was not wholly achieved. The 400 records included slightly more than a fair share which began from March to May and too few which

began in December and January. The 400 lactations provided 3,877 daily milk yields, using only the yields measured on the sixteenth of each month. Table 3 shows the analysis of variance between and within months.

The effect of month is unmistakably significant statistically but is not large enough to be of much practical importance, since the mean square is reduced only about 2.8 per cent by "holding month constant". The present results agree fairly well with those of Rhoad (5), as shown in table 4. The

TABLE 4
Mean daily milk yield by months

Month	Present study		Rhoad's study	
	Mean	Deviation of monthly mean from annual mean	Mean	Deviation of monthly mean from annual mean
	(l.)	(%)	(l.)	(%)
Jan.	4.31	+ 6.2	4.42	+ 10.0
Feb.	4.41	+ 8.6	4.57	+ 11.2
March	4.40	+ 8.4	4.42	+ 9.9
April	4.33	+ 6.6	4.23	+ 5.2
May	4.24	+ 4.4	3.94	- 4.5
June	3.96	- 2.5	3.63	- 9.7
July	3.77	- 7.1	3.62	- 10.0
Aug.	3.60	- 11.3	3.38	- 15.9
Sept.	3.60	- 11.3	3.62	- 9.9
Oct.	3.89	- 4.2	3.95	- 1.7
Nov.	4.14	+ 2.0	4.21	+ 4.7
Dec.	4.24	+ 4.4	4.42	+ 9.9
General mean	4.06		4.02	

effect of month is a bit more extreme in Rhoad's data, but the season of year when the yields are below average (after the dry season is well begun) is almost the same. Likewise, the maximum in both sets of data occurs from the middle to near the end of the rainy season. As one evidence that Rhoad's data show more extreme seasonal variations than the present data, the rate of decline from the highest month to the lowest by Brody's (1)

formula, $k = \frac{1n Y_2 - 1n Y_1}{t_2 - t_1}$, yields values of 0.050 and 0.034 per months for

Rhoad's data and for the present data, respectively. The difference can be attributed plausibly to the fact that the cows studied by Rhoad received no supplementary feed, while those used in the present study had some grass silage and some chopped sugar cane during the winter (dry) season. Also, it is possible that the few cases of twice-a-day milking in the present data were more frequent in the dry months, although that is not known for certain.

It seems worth emphasizing that the dry season in the tropics and sub-tropics appears to affect both milk production and growth, primarily through

its effect on the feed supply. This observation checks both with the present study and Rhoad's study and also with the results reported by Schutte (6) on the growth of beef cattle in South Africa. Schutte points out that rainfall rather than *season per se* causes the seasonal deviations, since the maximum growth of feed occurs approximately 3 months after the time of greatest precipitation.

Shape of the lactation curve. It is well known that under conditions of dairy management usual in the temperate zone, the lactation curve rises at the beginning of the lactation, reaching a maximum around 30 to 50 days after parturition, and then declines to the end of the lactation period. Whether this relation is the same under the penkeeping system was investigated by sorting the daily yields according to their order in the lactation. Yields measured in the first 14 days after parturition were called "first measurements", those from the 15th to the 29th day were called "second measurements", those from the 30th to the 44th day were "third measurements", and so on. This method of sorting placed all records with the same order of measurement in nearly the same segment of the lactation curve. Necessities of management, such as changing a cow from one *retiro* to another and accidents, of course resulted in failure to obtain some figures for

TABLE 5

Mean daily milk yields, in liters, at 15-day intervals from calving to the 30th measurement (450 days)

Order of measurement	No. of lactations	Mean yield	Order of measurement	No. of lactations	Mean yield	Order of measurement	No. of lactations	Mean yield
		(L.)			(L.)			(L.)
1	523	4.35	11	1316	4.18	21	579	3.01
2	1113	4.76	12	1313	4.12	22	482	2.95
3	1286	5.00	13	1308	4.04	23	379	2.85
4	1309	4.98	14	1290	3.94	24	288	2.75
5	1314	4.85	15	1264	3.85	25	288	2.75
6	1314	4.64	16	1201	3.67	26	174	2.63
7	1314	4.58	17	1125	3.51	27	136	2.65
8	1315	4.44	18	996	3.35	28	102	2.59
9	1315	4.37	19	836	3.24	29	75	2.58
10	1315	4.32	20	700	3.12	30	59	2.55

daily yields, especially those for yields at the first measurement date after parturition. The average number of days from calving to the first yield recorded was 18.6 ± 0.3 . Many of the cows began to go dry about the time of the 16th measurement (i.e., about 240 days), but a few continued through the 30th measurement. These averages are shown in table 5 and in figure 1.

Table 6 shows an analysis of variance within and between order of measurements. The effect of stage of lactation is statistically significant beyond all doubt and is large enough to be economically important, as somewhat more than 15 per cent of the variance between individual daily yields disappears when stage of lactation is held constant. The correlation be-

TABLE 6
Analysis of variance of daily milk yield between and within stages of lactation

Source of variation	<i>d/f</i>	Mean square	<i>F</i>
Total	25,968	2.74	
Between orders of measurement	29	38.21	16.5**
Within orders of measurement	25,939	2.32	

** = Highly significant.

tween yield and order of measurement was -0.198 . That this value was no larger numerically when the reduction in variance was over 15 per cent, of itself shows that the relation was not entirely linear. The straight regression lines shown in figure 1 were fitted separately. The rising one was fitted to the first three measurements and the declining one to the third to 30th measurements. The slope of the rising line is an average increase of 0.314 l. per 15-day interval, while the slope of the declining one is an average decrease of 0.107 l. per 15-day interval. Admittedly the sharp break in the curve at the third measurement is an artifact, resulting from the arbitrary division of the data at this point. The true curve probably sweeps

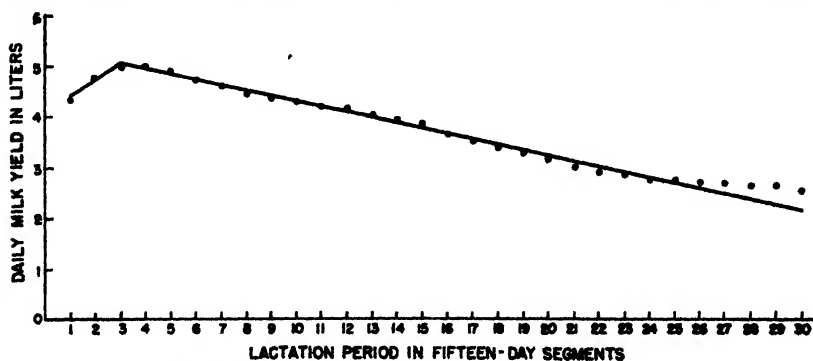


FIG. 1. Yields in successive stages of the lactation period.

smoothly with decreasing slope up toward a maximum and then slowly turns down again. However, as the choice of type of curve had to be arbitrary, further refinements to fit a single curve to the whole set of data did not seem worth while:

A straight line fits most of the declining part of the curve well until near the very end. There the numbers are few and it seems likely that the apparent lessening of the slope may be the result of selection, whereby those

which went dry and dropped out at each interval would mostly have been those which were producing the least at the preceding measurement. The rate of decline in the declining segment amounts to only 2.3 per cent each 15 days at the beginning of that portion, but is as much as 4.1 per cent of the current production by the time the lactation is about 12 months along. If the rate of decline from measurements 3-5 to measurements 24-26 is computed according to Brody's (1) formula (which, however, would make the regression a bit curvilinear), the decline is 2.86 per cent for each 15 days or about 5.7 per cent per month.

These rates of decline are not very different from those reported for dairy data from the type of management usual in temperate regions, although the means are much lower. Thus Brody (1) reports declines per month of 5.3, 5.5, 5.6, and 5.7 per cent, respectively, for groups of Holstein, farrow Guernsey, Jersey and Guernsey cows. It is somewhat surprising that the rates of decline should be so nearly the same in these data from a breed which is more nearly dual purpose, with cows milked only once a day, fed little but pasture, some of which was not very good, and suckled by their calves during the day. Possibly the management holds the production so much below the cow's inherent ability that this level of production remains more stable than it would under better management. However, this is only a tentative suggestion needing more investigation. Perhaps Brody's estimate that scrub cows decline almost 17 per cent per month needs testing on a wider variety of data. The progressive elimination of those which had been producing the least in each preceding month could explain the ap-

TABLE 7
Sex ratio among the calves

Year	Males	Females	Total
1930	149	116	265
1931	108	124	232
1932	130	113	243
1933	135	124	259
1934	129	106	235
1935	154	138	292
1936	173	154	327
1937	164	146	310
Total	1,142	1,021	2,163

parent slowness of the decline here after about the 15th measurement, but it could not explain the slowness of the earlier decline. There is no indication that such elimination is a noteworthy factor until about the 26th measurement.

Sex ratio. Although not a primary object of this study, the sex distribution by years was tabulated and is shown in table 7. Like most other reports on the sex ratio in cattle, this study shows a slight but statistically significant excess of males. The males comprised 52.8 per cent of the calves.

This compares with 51.8 reported by Crew (3), or the 50.5 by Gowen and Pearl, 49.4 by Roberts, 51.5 by Johansson, 52.2 by Ward, and 49.9 by Engeler, as quoted by Lush (4). The males were in excess every year except 1931 and in that year the difference was small. The statistical test for homogeneity from year to year yields a chi-square value of 5.42, which actually is a bit less than expected for seven degrees of freedom. Therefore, the conclusion is reached that the males were genuinely in excess of the females at Leopoldina, for reasons not known to the authors. This is in agreement with most other studies of the sex ratio in cattle. Whatever factor made the sex ratio depart from exact equality seems to have prevailed over all the years.

Heritability. Only a preliminary study of the heritability of individual differences in milk production has yet been made. The still unverified estimates are in the neighborhood of 0.5, which seems rather high as compared with most other studies. However, the herd contained cows of several different kinds of breeding. Some kinds contained distinctly higher percentages of dairy blood than others. This genetic heterogeneity would tend to make the heritability of differences within such a population higher than within a group which were all purebreds or high grades of the same breed. Also, the peculiarities of the penkeeping management, such as whether a cow would let down her milk freely to the hand milker in the morning when accustomed to being milked by the calf during the day, might make a noticeable difference. It is conceivable that the differences in behavior between the cows in this respect could be large and strongly hereditary. Speculation on this point seems unjustified until the existing evidence can be verified and examined from every point of view. The preliminary examination indicates a rather high level of heritability of individual differences in milk production under the penkeeping system.

SUMMARY

Records from a large farm in the *Zona da Mata* in Brazil were studied to learn about conditions which affect milk production under the penkeeping system.

Production varied significantly from year to year. Part of this is ascribed to an upward trend with time, but the statistical significance of that trend is not wholly assured. Some of the yearly means deviated rather widely from that trend.

Season of year had significant but rather small effects on the daily yield. The higher yields were toward the middle and end of the rainy season, while the lower ones were late in the dry season.

Production reached a maximum some time around 40 to 50 days after calving. Thereafter it declined in almost a straight line.

The sex ratio showed a slight but statistically significant excess of males. The year-to-year deviations of the sex ratio from the general mean were not statistically significant.

Heritability of individual differences in milk yield under these conditions seems moderately high.

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A COMPARATIVE STUDY OF THE BIOCHEMICAL ACTIVITY OF *STREPTOCOCCUS LACTIS*, *STREPTOCOCCUS CITROVORUS*, AND *STREPTOCOCCUS PARACITROVORUS* WHEN GROWN IN COW'S MILK AND SOYBEAN MILK

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The importance of acetyl-methylcarbinol and diacetyl from the standpoint of imparting a desirable flavor and aroma to butter and other food products is widely known. The value of selected cultures of bacteria for the development of these compounds has been well established through comparative studies on butter made with and without the use of butter cultures. Hammer and Babel (6) state in their review of butter cultures that previous to 1919 it was commonly believed that butter cultures were pure cultures of lactic acid streptococci, although there had been various suggestions that the desirable flavor of butter made from ripened cream was not produced by the lactic acid bacteria growing in the cream. In that year three laboratories established the basis for an understanding of the bacteriology of butter cultures by reporting almost simultaneously that such cultures normally include two distinct types of bacteria. Boekhout and Ott de Vries (1) isolated from sour milk and cream an organism which produced the characteristic and desirable butter culture aroma when grown with an organism of the *Streptococcus lactis* type; Hammer and Bailey (7) found that butter cultures contained organisms, associated with *S. lactis*, which commonly did not curdle milk but which in combination with *S. lactis* gave high volatile acidities; and Storch (14) considered two types of organisms necessary in butter cultures, a lactic acid type and a flavor type. The latter did not coagulate milk or form much acid but produced more volatile acid than the former. The two distinct types of organisms present in the butter cultures commonly used are *S. lactis* or *Streptococcus cremoris*, which primarily attacks the lactose and forms relatively large amounts of lactic acid together with small amounts of secondary products, and *Streptococcus citrovorus* and/or *Streptococcus*

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paracitrovorus, which are characterized mainly by the fermentation of citric acid to diacetyl and related compounds, some of which add greatly to the flavor and aroma of butter cultures, certain types of butter, cultured buttermilk, various cheeses and other food products. The numbers of bacteria in active butter cultures, as determined by plate counts, commonly are in the hundreds of millions and may be over 1 billion per ml. In the cultures studied by Hammer (4), *S. lactis* often made up 90 per cent of the flora and only occasionally fell under 75 per cent; in certain cases the flavor type made up only 1 to 3 per cent of the flora.

Price *et al.* (9) found that the lactic acid organisms usually produce from 0.7 to 1.0 per cent acid in milk, with the maximum about 1.2 per cent; most of the acid is lactic. Suzuki *et al.* (15) stated that the group of bacteria represented by *S. lactis* produces from 90 to 98 per cent of the theoretic yield of lactic acid from the sugar fermented, the remainder of the sugar going to alcohols, aldehydes and esters. With 50 cultures of lactic acid streptococci, Sherman and Albus (13) found the acid produced in milk (10 days at 35° C.) ranged from 0.60 to 0.95 per cent, the average being 0.80 per cent.

The high volatile acidities of butter cultures are evident from the odor and are readily detected by chemical procedures. Hammer and Bailey (7) found that the volatile acidities of butter cultures ranged from 31.2 to 37.6 (ml. of 0.1 *N* NaOH to neutralize 1 l. steam distillate from 250 g. culture); the total acidities varied from 0.87 to 1.08 per cent. Cordes and Hammer (3) noted that the volatile acidity of a butter culture increased as the total acidity increased until, in general, it reached 10 to 15 per cent of the total acidity. With 183 butter cultures grown in pasteurized milk, Templeton and Sommer (16) found the volatile acidities averaged 15.43 per cent of the total acidities; with 28 cultures grown in pasteurized milk plus 0.2 per cent citric acid, the value was 23.10 per cent. Boekhout and Ott de Vries (2) found that symbiosis of the two types of butter culture organisms yielded not only flavor but also considerable volatile fatty acid which was acetic.

The recognition by Van Niel *et al.* (17) that acetylmethylcarbinol (AMC) and diacetyl (AC₂) either are responsible for the aroma of butter or are the principal components of the aroma material soon led to studies on the production of AMC and AC₂ in butter cultures. Schmalfuss (10), by the sense of smell, detected AC₂ in a milk culture of a rod-shaped lactic acid organism and, through analyses, confirmed the identification of this compound. Van Niel *et al.* (17) noted that certain strains of propionic acid bacteria on a special medium (yeast-dextrose-chalk-agar) produced an odor similar to that of a high quality butter. A wide variation in the production of this odor was noted among the strains of propionic acid

bacteria, and a study of the products formed indicated that AMC was related to the typical butter aroma. It also was observed that a dilute aqueous solution of AMC or AC_2 had an odor characteristic of butter. The authors concluded that AC_2 either is responsible for the aroma of butter or is the principal component of the aroma material. Michaelian *et al.* (8) determined the amounts of AMC + AC_2 in satisfactory cultures and also in cultures lacking flavor. The results showed that considerable AMC plus AC_2 was present in satisfactory cultures. The authors also found that cultures contained only small amounts of AMC + AC_2 during the early stages of ripening, while conspicuous increases occurred later. It was observed that early in the ripening pronounced changes in titratable acid or pH had little effect on the amount of AMC + AC_2 present, but later striking increases occurred with little or no change in acidity. Hammer (5) and Michaelian *et al.* (8) made an extensive study of the relationship of AMC and AC_2 to butter cultures. Hammer and Babel (6) compiled an extensive review on the bacteriology of butter cultures. Schmalfuss and Barthmeyer (11, 12) studied the presence of AMC and AC_2 in various food materials and noted that the foods examined contained much more AMC than AC_2 .

Much research has been done on the study of flavor and aroma compounds in milk, butter, foods and other materials. However, to the authors' knowledge, no information has been published on the production of these compounds in soybean milk and its products. This investigation was undertaken to determine the comparative biochemical activity of the butter culture organisms, *S. lactis*, *S. citrovorus*, and *S. paracitrovorus*, in cow's milk and soybean milk.

Soybean or vegetable milk is used extensively throughout Japan and China for infant feeding as well as a food for adults. The introduction of soybean milk to the American people has occurred only recently. Soybean milk has been manufactured in the form of a powder. It has been used with good results in breads and cakes, in creaming vegetables, in custards, in chocolate or cocoa, and in several other food products as a substitute for cow's milk, especially in those countries that find it cheaper to use a vegetable milk. The high nutritive value of soybean milk and its many potential uses indicate that this product will continue to rise in importance as an item in the human diet. The development by butter culture organisms of AC_2 and related compounds in vegetable milk and products made from it may be desirable from a commercial standpoint.

EXPERIMENTAL PROCEDURE

The butter cultures used in this investigation were obtained from the Department of Dairy Industry, Iowa State College, Ames. The cultures

were carried in sterile skimmed milk, transferred daily, and incubated at 21° C. until coagulation occurred. The cultures were removed immediately after coagulation and held in the refrigerator at a temperature of approximately 10° C.

Seven-hundred-milliliter samples of skimmed cow's milk and of soybean milk were placed in quart milk bottles, plugged with rubber stoppers, covered with wrapping paper, and sterilized by heating to 100° C. for 20 minutes on 3 consecutive days. The soybean milk used was obtained from Harry Miller, Director of the International Nutrition Laboratory, Mt. Vernon, Ohio. The sterilized samples of milk were inoculated with butter culture organisms (3 ml.) and held at 21° C. for 0, 12, 28, 48, 72, 96, 168 and 216 hours. The hydrogen-ion concentration, titratable acidity, volatile acidity, and $\text{AMC} + \text{AC}_2$ were determined in duplicate on each sample at the end of each incubation period.

The pH determinations were made on 10-ml. samples of each culture of fermented milk, using a Coleman 3C glass electrode potentiometer.

The titratable acidity was determined by the titration of 10 ml. of the culture with 0.1 *N* sodium hydroxide, using phenolphthalein as the indicator. The end-point taken was that point at which a faint pink color remained for 1 minute. The acidity obtained was expressed as per cent lactic acid.

The volatile acidity was determined by the method of Michaelian *et al.* (8). Two hundred and fifty grams of the cultured milk with 250 ml. of distilled water was steam distilled after the addition of 15 ml. of *N* sulfuric acid. The first 1,000 ml. of distillate was titrated, using 0.1 *N* sodium hydroxide and phenolphthalein. The results were expressed as the ml. of 0.1 *N* sodium hydroxide required to neutralize the acidity. In determining the amounts of flavor and aroma compounds by the procedure of Michaelian *et al.* (8), a 200-g. portion of the milk was distilled with steam after adding 40 ml. of ferric chloride solution to oxidize the AMC to AC_2 . Hydroxylamine hydrochloride, sodium acetate, and nickel chloride solutions were added to the distillate as a mixture. The material was allowed to stand at least 1 day in order to permit complete crystallization; the nickel salt then was filtered into a weighed crucible. The salt was washed with distilled water, dried to constant weight at 110° C., and the results were expressed as the milligrams of nickel dimethylglyoximate per 200 g. of cultured milk.

RESULTS AND DISCUSSION

The values obtained for the pH and volatile acidity were comparable in most instances for both types of milk held at the various incubation periods. After cultures of fermented soybean milk were held for 96 to 216 hours, the volatile acidities ranged from 24.2 to 32.3 (ml. of 0.1 *N* NaOH to neutralize 1 l. of steam distillate from 250 g. of culture);

TABLE 1

The relationship between certain biochemical activities of better culture organisms when propagated in cow's and soybean milk (Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

	Hours incubated								
	0	4	12	24	48	72	96	168	216
Cow's milk									
pH	6.6	6.1	5.7	4.3	4.3	4.3	4.4	4.4	4.3
Titratable acidity ^a	0.15	0.20	0.25	0.83	1.12	1.07	1.04	1.08	1.03
Volatile acidity ^b	4.1	4.0	4.1	9.6	20.4	23.5	23.4	33.8	27.5
Mg. Ni salt ^c	None	None	Trace	10.2	18.6	15.3	18.7	18.2	16.9
Soybean milk									
pH	6.1	5.7	5.4	4.6	4.5	4.5	4.5	4.5	4.4
Titratable acidity ^a	0.17	0.17	0.24	0.45	0.49	0.51	0.55	0.49	0.51
Volatile acidity ^b	3.8	6.4	7.1	10.5	12.5	10.9	24.2	25.0	32.3
Mg. Ni salt ^c	None	None	None	None	4.6	9.3	12.5	33.6	21.4

^a As per cent lactic acid per 10 cc sample.

^a As per cent lactic acid per 10 g. sample.

^b Values expressed in ml. of 0.1 N sodium hydroxide per 1,000 ml. of distillate.

^c Milligrams of nickel dimethylglyoximate per 200 g. of culture.

whereas, for cow's milk the values were 23.4 to 27.5 ml. (table 1). The value for the volatile acidity in cow's milk plus 0.15 per cent citric acid averaged 32.6 ml. for an incubation period of 96 to 216 hours; the corresponding value for cultured soybean milk was 33.5 ml. (table 2).

The production of AMC plus AC_2 in soybean milk by butter culture organisms was not evident until after an incubation period of 48 hours; however, upon incubation of cultured soybean milk for 168 to 216 hours, larger amounts of the flavor and aroma compounds were produced in soybean milk as compared to cow's milk (table 1). When the samples were held for 168 hours at 21° C., 33.6 mg. of Ni salt were obtained from 200 g. of cultured soybean milk. In cow's milk held under similar conditions, 18.2 mg. of Ni salt were obtained. In cow's milk a trace of AMC plus AC_2 was formed after a 12-hour incubation period. The results obtained for the production of AMC plus AC_2 in cow's milk are in agreement with those of Michaelian *et al.* (8), who found that satisfactory cultures yielded 10 mg. or more nickel dimethylglyoximate per 200 g., the maximum being 39.5 mg. The results obtained for the cultured soybean milk are comparable.

When the butter culture organisms were grown in cow's milk plus 0.15 per cent citric acid, the AMC plus AC_2 content was found to the extent of 14.2 mg., as the Ni salt, per 200 g. of culture after an incubation period of 12 hours (table 2). The AMC plus AC_2 content increased to 44.7 mg. upon incubation of the cultures for 216 hours. For cultured soybean milk plus 0.15 per cent citric acid the production of AMC plus AC_2 was retarded; 2.4 mg. of the Ni salt were obtained after an incubation period of 24 hours, which increased to 42.3 mg. after 216 hours of incubation. AMC plus AC_2 was not developed as rapidly in soybean milk as in cow's milk, but upon prolonged incubation the results obtained were in close agreement.

The AMC plus AC_2 content of samples of cultured cow's milk and soybean milk held for 72, 96, 168, and 216 hours at 21° C. averaged 17.2 and 19.2 mg. of the Ni salt, respectively (table 1). When 0.15 per cent citric acid was added to the cultures, the average values obtained were: cow's milk, 33.2 mg.; soybean milk, 32.5 mg. (table 2). This represents a 93 and 70 per cent increase in the AMC plus AC_2 content of the cultured cow's milk and soybean milk, respectively.

The values obtained for the titratable acidity in cow's milk and cow's milk to which 0.15 per cent citric acid was added were nearly twice as great as those secured for the cultured soybean milk. Early in the ripening pronounced changes in titratable acid or pH had little effect on the amount of AMC plus AC_2 present, but later significant increases occurred with little or no change in acidity. These results are in agreement with those of Michaelian *et al.* (8).

TABLE 2

The relationship between certain biochemical activities of butter culture organisms when propagated in cow's and soybean milk modified by the addition of citric acid

(Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

	Hours incubated,									
	0	4	12	24	48	72	96	168	216	
Cow's milk (0.15 per cent citric acid added)										
pH	6.6	6.2	4.8	4.8	4.2	4.0	4.0	4.4	4.4	
Titratable acidity ^a	0.15	0.21	0.90	0.90	1.04	1.08	1.13	1.15	1.10	
Volatile acidity ^b	4.3	4.4	10.8	16.1	33.6	27.5	33.5	31.0	33.5	
Mg. Ni salt	None	None	14.2	36.4	32.0	30.3	28.6	29.5	44.7	
Soybean milk (0.15 per cent citric acid added)										
pH	6.1	5.6	4.8	4.8	4.3	4.2	4.0	4.5	4.5	
Titratable acidity ^a	0.16	0.15	0.51	0.45	0.64	0.66	0.68	0.64	0.67	
Volatile acidity ^b	4.0	6.6	10.8	9.1	20.2	21.2	31.3	31.5	37.7	
Mg. Ni salt	None	None	Trace	2.4	12.6	36.4	20.9	30.4	42.3	

^a As per cent lactic acid per 10 g. sample.

^b Values expressed in ml. of 0.1 *N* sodium hydroxide per 1,000 ml. of distillate.

^c Milligrams of nickel dimethylglyoximate per 200 g. of culture.

Further data are presented in table 3 for samples of cultured soybean milk to which 0.10 0.15, 0.20, and 0.30 per cent citric acid was added.

TABLE 3

The relationship between certain biochemical activities of butter culture organisms when propagated in cow's milk and soybean milk re-enforced with citric acid

(Av. of two determinations. Cultures grown in 700 ml. of sterile substratum inoculated with 3 ml. of culture and incubated at 21° C.)

Hours held at 21° C.	Added citric acid (%)	pH	Titratable acidity ^a	Volatile acidity ^b	mg. of Ni salt ^c
Soybean milk					
0	0.10	6.1	0.17	3.8	None
48	0.10	4.6	0.68	11.5	11.9
72	0.10	4.6	0.62	23.0	23.4
120	0.10	4.3	0.64	33.5	40.1
0	0.20	6.1	0.17	3.8	None
48	0.20	4.6	0.67	12.3	15.0
72	0.20	4.7	0.70	27.0	47.0
120	0.20	4.4	0.77	40.0	44.0
72	0.15	4.4	0.65	21.1	20.3
120	0.30	4.4	0.66	40.2	35.4
Cow's milk					
72	0.15	4.3	1.02	39.3	49.1
120	0.30	4.3	1.04	40.7	46.0

^a As per cent lactic acid per 10 g. sample.

^b Values expressed in ml. of 0.1 N sodium hydroxide per 1,000 ml. of distillate.

^c Milligrams of nickel dimethylglyoximate per 200 g. of culture.

SUMMARY AND CONCLUSIONS

A comparative study was made of pH, titratable acidity, volatile acidity, and acetylmethylcarbinol plus diacetyl on samples of cow's milk and soybean milk inoculated with the butter culture organisms, *Streptococcus lactis*, *Streptococcus citrovorus* and *Streptococcus paracitrovorus*.

The values secured for the pH and volatile acidity were comparable in most instances for both types of milk held at the various incubation periods. The value for the volatile acidity in cultured cow's milk averaged 28.3 (ml. of 0.1 N NaOH to neutralize 1 l. of steam distillate from 250 g. of culture) for an incubation period of 96 to 216 hours; the corresponding value for cultured soybean milk was 27.2 ml. For cultured cow's milk and soybean milk plus 0.15 per cent citric acid, the values were 32.6 and 33.5 ml., respectively.

During the early stages of fermentation of cultured cow's milk and

soybean milk, only small amounts of acetylmethylcarbinol plus diacetyl were present, while after 96 hours of incubation at 21° C. appreciable increases in these substances occurred.

Samples of cultured soybean milk held 168 to 216 hours contained larger amounts of acetylmethylcarbinol plus diacetyl than cultured cow's milk; 33.6 mg. of Ni salt were obtained from 200 g. of cultured soybean milk held 168 hours at 21° C.; whereas, in cow's milk held under similar conditions, 18.2 mg. were found.

The acetylmethylcarbinol plus diacetyl content of eight samples of cultured cow's milk and eight samples of soybean milk held 72 to 216 hours at 21° C. averaged 17.2 and 19.2 mg. of the Ni salt, respectively. When 0.15 per cent citric acid was added to the cultures, the values obtained were: cow's milk, 33.2 mg.; soybean milk, 32.5 mg. The addition of citric acid resulted in a 93 and 70 per cent increase in the production by butter culture organisms of acetylmethylcarbinol plus diacetyl in cow's milk and soybean milk.

Acetylmethylcarbinol plus diacetyl was not developed as rapidly in soybean milk as in cow's milk, but upon extended holding of the cultures the results obtained were comparable.

Cultures of cow's milk held at 21° C. for 72 to 216 hours had an average lactic acid content of 1.05 per cent; whereas, cultures of soybean milk held under similar conditions had a lactic acid content of 0.51 per cent. Samples of fermented cow's milk plus 0.15 per cent citric acid held for 72 hours or longer contained nearly twice as much lactic acid as samples of fermented soybean milk.

Early in the ripening pronounced changes in titratable acid had little effect on the development of acetylmethylcarbinol plus diacetyl in cow's milk and soybean milk, but later in the incubation period significant increases occurred with little or no change in acidity.

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THE ROLE OF SURFACE-ACTIVE CONSTITUENTS INVOLVED IN THE FOAMING OF MILK AND CERTAIN MILK PRODUCTS.

III. MILK LIPIDS, INCLUDING PHOSPHOLIPIDS¹

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The role of the milk lipids in the foaming of milk is not clearly defined. Van Dam (27), by decreasing the fat content of separated milk from 0.22 to 0.08 per cent, and Mohr and Brockmann (14), by increasing it from 0.04 to 1.0 per cent, concluded that the foaming capacity and foam stability varied inversely with the fat content. Sommer and Horral (24) found addition of fat to a skim milk-gelatin-sugar mix greatly decreased whipping ability. El-Rafey and Richardson (5) attributed the minimum foaming of skim milk, whey, lactalbumin sols, and blood serum at approximately 27° C. to the presence of fat globules.

Leete (11), on the other hand, from studies with skim milk, milk, and cream, concluded no definite statement could be made regarding the effect of milk fat on foaming without considering temperature. Sanmann and Ruehe (22), failing to find a definite relationship between the fat or solids content of milk and its foaming ability, suggested that the foaming ability of milk from individual cows largely is dependent upon factors which are characteristic of the cow. When these latter factors were controlled, they found that increasing the fat content usually decreased the foaming ability; the reverse was true with respect to solids-not-fat. According to Holm (7), increases in the fat content of milk over that normally present result in increased foaming and great foam stability.

The effect of the physical state of the fat on its influence on foaming has been recognized. Mohr and Brockmann (14) found that milk exhibits greater foaming properties at temperatures at which the fat is liquid than where it is solidified. This appears to be contrary to the theory proposed by Leviton and Leighton (12) that the destructive action of milk fat and other lipids on foam depends upon their ability to spread on water. It has been reported that the concentration of fat in sodium caseinate solutions is of less significance on their foaming capacity than the chemical and physical condition of the fat (19).

Preliminary studies of the role of milk fat in the foaming of milks and

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creams suggested not only that the concentration, the chemical composition, and the physical state of the fat must be considered, but also that the nature of the material comprising the interface between the fat globules and the aqueous medium is of even greater significance.

EXPERIMENTAL PROCEDURE

The method of measuring foaming capacity and foam stability has been described in a previous publication (4). The milk fat used in this study was obtained by churning fresh cream from mixed herd milk, melting the butter at 50° C., and filtering the decanted fat at the same temperature. The fat was stored at 5° C.

Preliminary investigations showed that soybean phospholipids gave essentially the same results when used as emulsifiers as milk phospholipids isolated from separator slime. Soybean phospholipids,⁴ which are a mixture including lecithin, cephalin, and probably inositol phosphatide, were used in these studies.

The natural emulsions were prepared by diluting cream with the appropriate volumes of its separated milk. The artificial emulsions were prepared by dispersing the fat or oil, with or without added phospholipid, in pasteurized separated milk, or other medium, using a two-cylinder hand emulsifier.⁵ When stabilizers, such as gum arabic, were used, the fat first was triturated with the powdered gum and distilled water, and the resulting cream, after being diluted to the desired concentration, was passed through the emulsifier.

The size of the fat globules in the creams was determined microscopically using the technic of Cole and Smith (3). Emulsification was considered satisfactory when the globules ranged in diameter from 1 to 11 μ , with an average diameter of 3.5 μ . To attain this it sometimes was necessary to pass the mixtures through the emulsifier three to five times.

Phosphorus in the fats was determined colorimetrically (31), the fat being ashed according to the method of Horrall (8).

RESULTS

Effect of fat percentage on the foaming of milk and cream. At certain temperatures, at least, skim milk and cream yield their own distinctive type of foam, as illustrated in figure 1. In a series of milks and creams of increasing fat contents, both types of foam will be expected to be present at a certain fat content, and somewhere in the series a reversal of predominating types will take place.

⁴ "Margo", 70 per cent phospholipid in soybean oil, courtesy of Dr. J. Eichberg, American Lecithin Company, Long Island City, N. Y. "Best Grade Lecithin", courtesy of Mr. D. C. Ingraham, Durkee Famous Food Company, Berkeley, California.

⁵ Club Aluminum Products Co., Chicago, Illinois.

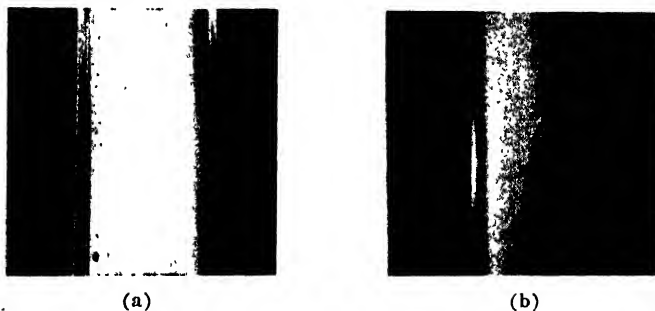


FIG. 1. Types of foam from milk and cream. (a) Skim milk or protein type. (b) Cream or lipoprotein type.

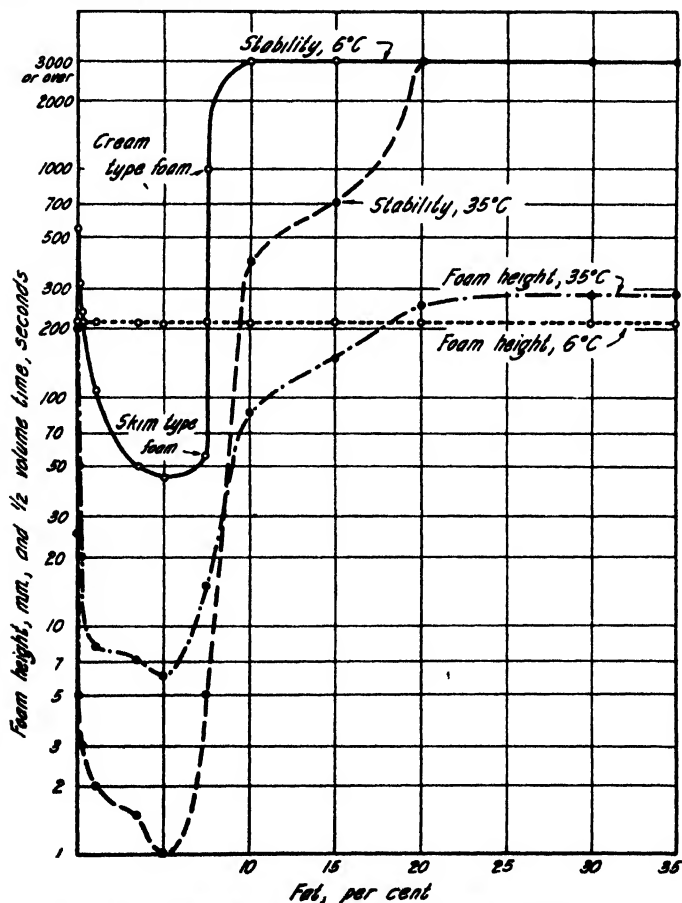


FIG. 2. The effect of increasing the fat content of skim milk from 0.01 to 35.0% on its foaming properties at 6° C. and at 35° C.

A series of samples ranging in fat contents from 0.01 to 35.0 per cent was prepared using raw separated milk and raw cream. As shown in figure 2, at both 6 and 35° C., as the fat content increases, the foam stability first decreases to a minimum value, and this is followed by a rapid increase in stability. With fat concentrations up to between 5.0 and 7.5 per cent, the foam consisted of small, compact cells with a short half-volume time. Above 7.5 per cent of fat, a cream-type or lipoprotein-type of foam predominated. This latter consisted of large cells with distorted lamellae that maintain an increasingly stable structure with increasing fat percentage.

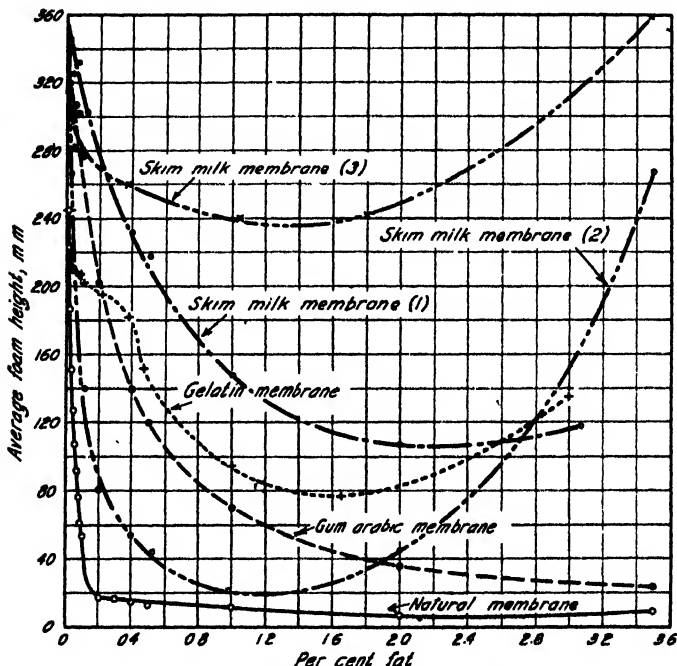


FIG. 3. The effect of the type of stabilizer on the foaming of milk fat emulsions at 35° C. (All emulsions were made by diluting creams, stabilized as indicated, with skim milk. The emulsions for curve (2) differ from those of (1) in that the fat for (2) contained 4% phospholipids added prior to emulsification. Curve (3) represents emulsions of soybean oil containing 4% added phospholipids.)

At 6° C. both types of foam were visible in the 7.5 per cent milk. At 6° C. the foam volume remained constant regardless of the fat content.

Effect of the emulsifying agent on foaming. Various emulsions were prepared as follows: (a) Natural raw cream was diluted with raw separated milk to give a series of milks ranging in fat contents up to 3.5 per cent. (b) Artificial emulsions were made by emulsifying milk fat into raw separated milk, gelatin sols, or gum arabic and diluting the resulting

creams with separated milk to give a series of milks of fat contents similar to those in (a). (c) Artificial emulsions were made by emulsifying milk fat or soybean oil, each containing 4 per cent added soybean phospholipids, into raw separated milk and diluting these with the separated milk to obtain the series of milks of varying fat contents. The foaming characteristics of these milks at 35° C. were studied.

The results are shown in figure 3. With the emulsions made with the natural cream, a rapidly progressive decrease in foaming occurs with increasing fat content up to 0.2 per cent fat, after which no marked further decreases occur. The emulsions of milk fat containing the added phospholipids showed a marked resemblance to the natural emulsions up to a fat content of 1 per cent. With increasing fat contents, however, the foaming capacity (and foam stability) increased. The type of this latter foam was distinctive. It was coarse in structure, consisting mostly of five- and six-membered rings. It exhibited a marked glistening and iridescent appearance. The lamellae became very thin and the whole structure collapsed in an explosive manner. This type of foam will be referred to as the "phospholipid" foam. As later experiments will indicate, the 4 per cent added phospholipid is excessive.

The effect of increasing fat contents is not so marked in those emulsions stabilized with skim milk or gelatin. No changes in the nature of the foams were observed with increasing fat contents. With the gum arabic-stabilized emulsions, increasing concentrations of fat exerted a slow but progressive depressing action on foaming. The depressing action of the soybean oil containing added phospholipids was found to be less marked than that of milk fat, but, in the higher concentrations, the typical phospholipid foam was observed.

Effect of increasing phospholipid content on the foaming of separated milk. A 2 per cent soybean phospholipid emulsion, prepared by dispersing it in pasteurized skim milk at 50° C. and passing it five times through the emulsifier, was diluted with the skim milk to give a series ranging from 0.0 to 2.0 per cent added phospholipid. Foaming tests were made at 21.5° C. The results are shown in figure 4. As the added phospholipid increases to 0.05 per cent, the foaming tendency decreases. This decrease is about six times that obtained by adding soybean oil in amounts equivalent to that associated with the added phospholipid.

As the concentration of phospholipid increases beyond 0.05 per cent, a typical phospholipid foam begins to appear, first as a coarse unstable foam, similar to that of buttermilk, and followed by foams of increasing height, compactness, iridescence and stability.

Effect of phospholipid concentration in the fat on foaming of milk emulsions. Four 10 per cent fat emulsions were prepared by emulsifying,

in pasteurized skim milk, milk fat containing 0.0, 0.45, 0.7, and 1.0 per cent added soybean phospholipid. The foaming properties were studied at various temperatures between 5 and 55° C. Figure 5 shows that each emulsion yielded foams of minimum stability at temperatures between 27 and 35° C. As the phospholipid content increased, the protein-type foam, characteristic

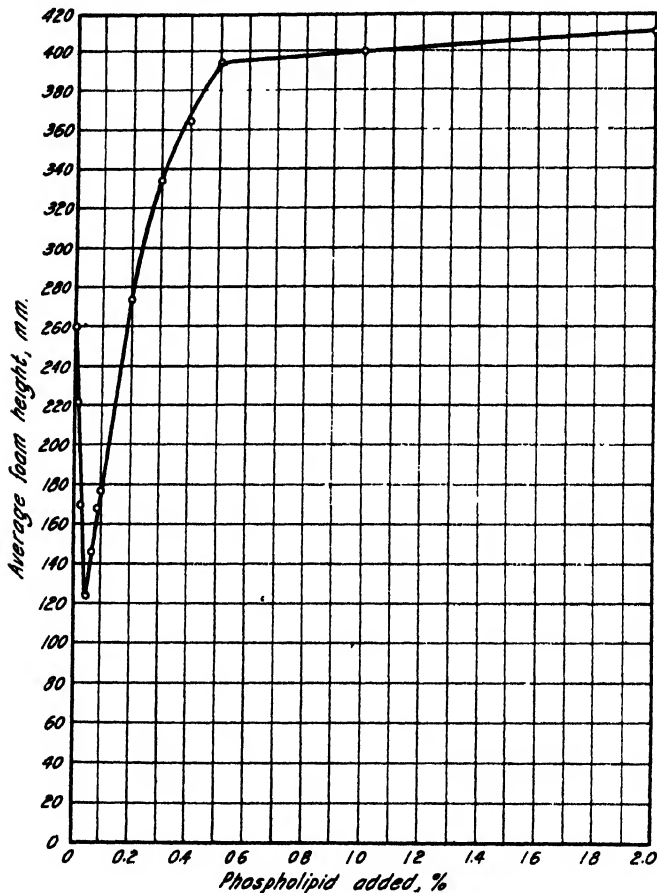


FIG. 4. The effect of added soybean phospholipids on the foaming of skim milk at 21.5° C.

of skim milk, gradually was replaced by a cream-type or lipoprotein-type foam.

No further increases in foaming capacity or foam stability occurred at temperatures above 50° C. In figure 6 it is seen that the emulsions made with fat containing about 0.8 per cent phospholipid yielded foams of minimum or low stabilities at all the temperatures below 50° C. The artificial

emulsions made with fat containing from 0.5 to 1.0 per cent phospholipid had foaming characteristics more closely resembling those of the natural emulsion made from cream and skim milk. At 23° C. the emulsions pre-

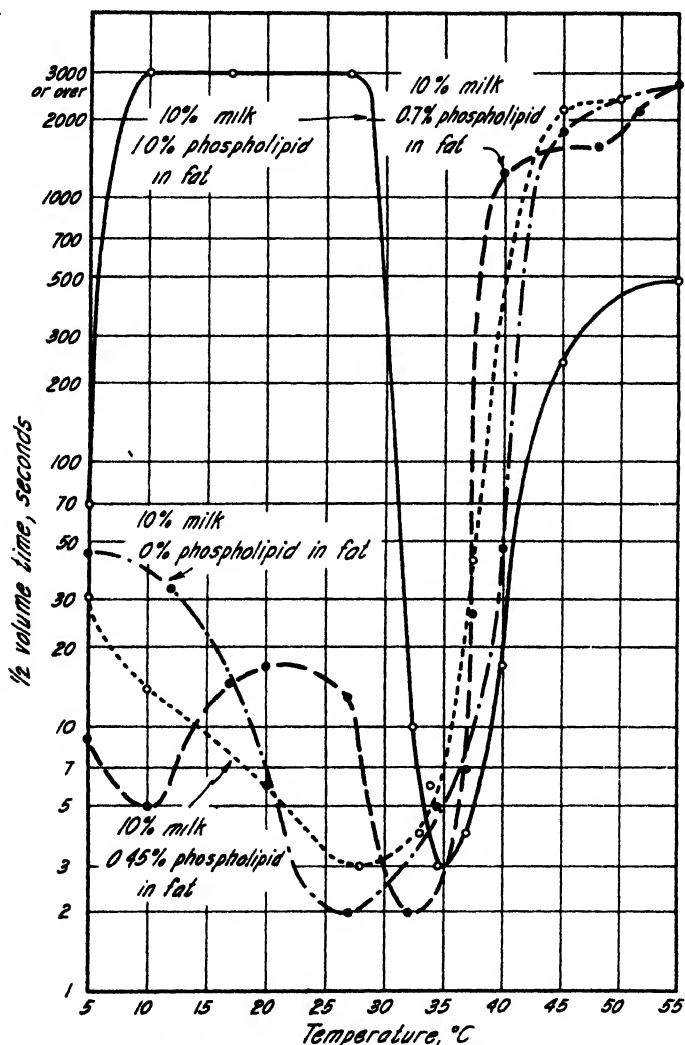


FIG. 5. The effect of temperature on the stability of foams of 10% emulsions of milk fat, containing varying concentrations of soybean phospholipids, dispersed in pasteurized skim milk.

pared with fat containing 0.8, 0.85, and 0.9 per cent phospholipid showed two types of foam, a protein foam that subsided quickly and the very stable cream-type of foam.

Effect of increasing the concentration of the fat of constant phospholipid content on the foaming of artificial creams. Artificial creams were prepared by emulsifying milk fat containing 0.5 per cent added soybean

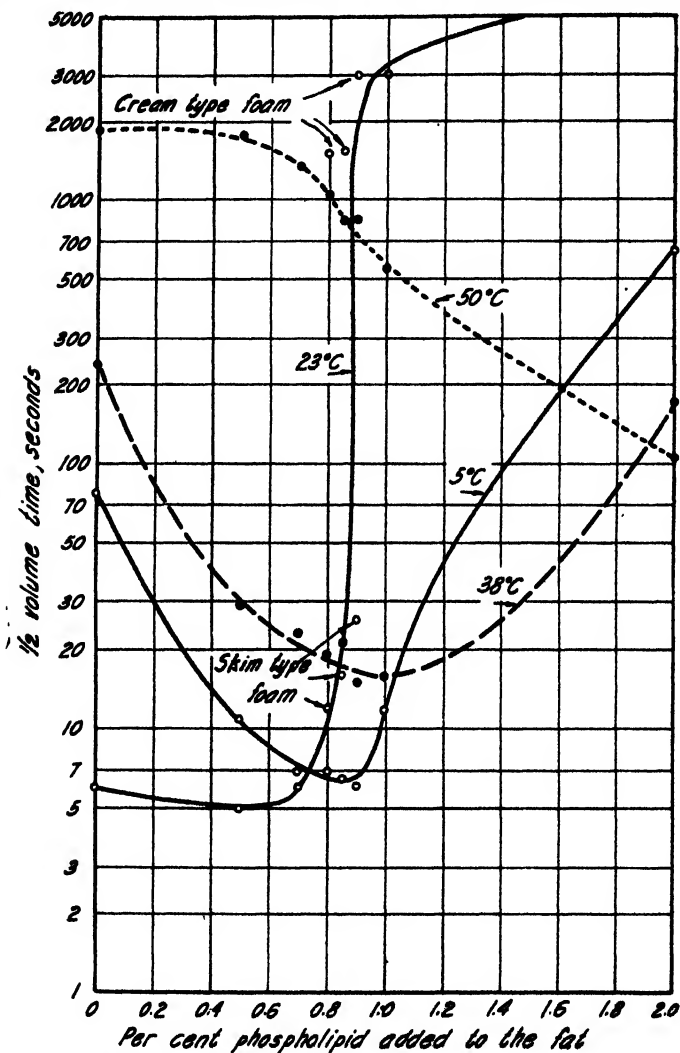


FIG. 6. The effect of increasing the phospholipid content of milk fat from 0.0 to 2.0% on the foam stabilities of a 10% emulsion in pasteurized skim milk at 5, 23, 38 and 50° C.

phospholipid into pasteurized skim milk. Natural creams were included. The results, shown in table 1, indicate that at 5 and 28° C. the foaming

tendency and the foam stability increase as the fat concentration increases from 10 to 40 per cent; the surface tension decreases with increasing fat concentration. At 50° C. the foaming capacity remains constant for increasing fat concentration, while the surface tensions and the half-volume times decrease progressively with increases in the fat. The surface tension values and the foaming properties of the 30 per cent artificial cream were very similar to those of the pasteurized natural cream of similar fat content.

Effect on foaming properties of emulsifying milk fat, with and without added phospholipids, in buttermilk. Wiese and Palmer (30) found that remade milk, made by dispersing butterfat in buttermilk, closely resembled whole milk in many respects, including the churnability of its cream. On

TABLE 1

The effect of concentration of fat of constant phospholipid content on the foaming of artificial creams

Sample	Results at 5° C.			Results at 28° C.		
	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
10% milk		115	12	43.4	40	5
20% cream		162	17	45.0	100	> 3000
30% cream		200	1220	42.3	240	> 3000
40% cream		205	> 3500	40.0		
31% natural cream	51.0	200	> 3000	42.7	240	> 3000

the other hand, emulsions made by dispersing milk fat in calcium caseinate, lactalbumin, lactoglobulin, or phospholipid-aqueous media behaved quite abnormally. These investigators used milk fat in which the phospholipid presumably would be absent or nearly so. They did not add phospholipid to the fat.

Two creams were made by emulsifying milk fat or milk fat containing 0.5 per cent soybean phospholipid into buttermilk from sweet, pasteurized cream. Foaming was studied at three temperatures and the results compared with those obtained with a 31 per cent pasteurized natural cream. The results (table 2) show that the addition of phospholipid to the milk fat prior to emulsification improves the foaming properties of the artificial creams to the extent that they resemble those of natural cream. The foam stability of these creams at 5° C. and 23° C. is almost eight times that of the cream with no phospholipid added to the fat.

It appears to have been established that the natural fat globule "membrane" of milk and cream is some sort of phospholipid-protein com-

plex. Palmer (15) stated, "Indeed, not only was it found that the emulsion properties and churnability of artificial emulsions of milk fat in the various colloidal sols from milk plasma are strikingly different from those of washed natural cream but also that the 'membrane' materials isolated from the washed artificial creams are also chemically distinct from the natural 'membrane' substances". To the authors' knowledge no attention has been paid to the presence or absence of phospholipid dissolved in the fat being emulsified. It is known, however, that the amount of these compounds left associated with the fat after its isolation depends upon the method of

TABLE 2

*The foaming of 30% creams prepared by emulsifying butterfat, with and without 0.5% added soybean phospholipids, in buttermilk from sweet cream containing 31% fat**

Cream	Results at 5° C.			Results at 23° C.		
	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
30% cream in buttermilk, no added phospholipid	54.4	200	396	48.2	220	435
30% cream in buttermilk, 0.5% phospholipid added to the fat	52.3	200	> 3000	43.5	240	> 3000
31% natural cream	51.6	200	3000	42.9	240	> 3000
Buttermilk (0.4% fat)	51.9	170	20	47.1	60	25

* At 50° C. all samples gave the protein-type foam.

isolation (6, 21). Jenness and Palmer (9) did have some indication that the protein as it is "eroded" during churning pulls away varying amounts of phospholipid with it. Observations in this laboratory (18) revealed striking visible differences at the interface between liquid milk fat superimposed upon a warm aqueous medium depending upon whether or not the fat contained phospholipid and whether or not the medium was pure water or contained whey proteins. The interface between the fat containing phospholipid and the pure water was cloudy, indicating solvation of the phospholipid; all the other interfaces remained clear.

The effect of incorporation of phospholipids in the fat of artificial creams on some of their physical properties. Soybean phospholipids were

dissolved in milk fat to a concentration of approximately 1.0 per cent by heating to 50° C. The solution was clear but showed a tendency to give an iridescent-type foam. A 25 per cent dispersion was prepared by emulsifying it in distilled water at 45° C. The emulsion was diluted to give creams containing 16.5 per cent butterfat, one-half being diluted with distilled water and the other half with a 0.5 per cent lactalbumin solution. The lactalbumin was isolated as described previously (4). Both creams were held overnight at 2° C.

TABLE 3

Foaming properties of creams prepared with butterfat with 1.0% soybean phospholipid added emulsified in distilled water and in 0.5% lactalbumin solution

Temp.	Surface tension	Foam height	½ volume time	Surface tension	Foam height	½ volume time
	16.5% cream (1% phospholipid in fat) in distilled water			16.5% cream (1% phospholipid in fat) in 0.5% lactalbumin		
(°C.)	(dynes/cm.)	(mm.)	(sec.)	(dynes/cm.)	(mm.)	(sec.)
5	47	190	250	48.9	210	> 3000 ^a
16	40.9	40	10	46.6	185	1450 ^a
25	40.5	20	6	40.5	30	10
37	29.1	100	> 3000 ^b	29.6	15	5
45	29.1	105	> 3000	29.0	15	5
	Buttermilk from above cream 0.61% fat			Buttermilk from above cream 1.1% fat		
5	46.5	5	2	45.4	70	8
8	—	3	2	—	50	5
19	39.6	2	2	45.2	10	3
37	36.7	15	4	41.0	2	1
45	31.5	15	4	35.2	8	1.5

^a Typical cream-type foam.

^b At 37° C. the cream oiled off and an iridescent phospholipid foam originated from the oil layer on top.

Upon examination, the cream prepared in water contained large fat aggregates which stuck to the walls of the container and resembled those formed when normal cream is on the verge of "breaking" during churning. On heating to 37° C., the emulsion oiled off. Churning time at 10° C. was about 5 minutes. The butter formed a solid crumbly mass and the buttermilk contained 0.61 per cent fat (Babcock). The other cream prepared in the albumin solution had a smoother body, less tendency for fat aggregation, and a longer churning time (about 12 minutes). The butter was more

plastic, the butter granules maintained their individuality, and the buttermilk contained 1.1 per cent fat. The cream formed a stable cream-type foam between 5 and 15° C., and the foam decreased in volume and stability at increased temperatures.

Table 3 shows that the surface tensions of the creams were very similar except at the temperature of 16° C.; those of the buttermilk from the lactalbumin cream were higher than those of the other except at 5° C. The foaming characteristics of the creams and the buttermilks from them were diametrically different with respect to the effect of temperature. The lactalbumin-phospholipid stabilized cream gave the typical cream-type foam at low temperatures; the protein-free cream yielded the typical phospholipid foam at the higher temperatures.

TABLE 4

Phosphorus in the filtered butterfat from butter churned from artificial creams

Sample no.	Description	Weight of fat ashed	Total phosphorus	Phosphorus
		(g.)	(g.)	(mg./g. fat)
1	Original fat	4.1527	0	0
2	Original fat plus approx. 1% phospholipids	4.0591	0.855	0.211
3	Fat from butter of cream from fat no. 2 in distilled water	4.0365	0	0
4	Fat from butter of cream from fat no. 2 in 0.5% lactalbumin solution	4.017	0	0

The analyses for phosphorus in the fat from the butter of each cream (table 4) showed that protein is not necessary to "pull" the phospholipid from the fat during churning. Apparently, the mere solvation of the polar phosphoric acid-choline group of the lecithin and of the phosphoric acid-ethanolamine group of the cephalin is sufficient.

DISCUSSION

The results of this study clarify, in some measure at least, the problem of making artificial emulsions of milk fat with properties similar to the natural product, using milk solids as stabilizers. Most workers in the past have overlooked the importance of incorporating phospholipids in the fat prior to emulsification. Wiese and Palmer (30) recognized that "The butterfat-in-buttermilk dispersion resembles whole milk in every respect, in general appearance, microscopic structure, cream separation and churnability of the cream." When they prepared stable emulsions with the

proteins of milk as emulsifiers, these emulsions were abnormal in one or more of their properties. The best churning was obtained with a phospholipid stabilizer. All stabilizers were incorporated in the aqueous phase prior to emulsification. Wiese *et al.* (29), in studies on the rebodilyng of cream, found that only those emulsions prepared with the normal fat globule membrane present during the emulsification responded to the rebodilyng process. They also noted that the composition of the butterfat appears to be a factor, presumably referring to the chemical constants of the fat, rather than its purity with respect to phospholipids. As stated earlier, the phospholipid content of filtered milk fat depends upon the purification procedure (6, 21). It is known also that the phospholipids of milk, cream, and butter exist in at least three states: free, loosely-bound to protein (probably by secondary valences), and chemically-bound to protein (primary valences) (2, 17, 26). Jenness and Palmer (9) emphasized that the ratio of phospholipid to protein was greater in the serum of washed-cream butter than in its buttermilk. It would be interesting to know the ratio of cephalin to lecithin in this respect, especially in view of the work of Spiegel-Adolph (25), Chargaff (2) and Rewald (17), who showed that the phosphatides of butter consisted of 50 per cent lecithin, 36 per cent cephalin, and 14 per cent other phosphatides.

Exact duplication of the foaming or other physical properties of a milk or cream by using artificial emulsions compounded from purified milk fat and other milk solids isolated by chemical means obviously is extremely difficult. The natural membrane material from cream which has not been washed too well would be expected to be the best emulsifier.

The authors postulate the following explanation, based on thermodynamical considerations. Assuming that milk fat is elaborated separately from the plasma solids (16), that the neutral blood fat is the main precursor of milk fat, and that blood phospholipids or phospholipid-protein complexes take part in its transfer, it seems logical to assume that, initially, milk fat contains, or is associated with, phospholipids, free and/or linked to protein. These, being surface-active, would tend to migrate to the interfaces between the fat and the aqueous medium during globule formation, their relative concentration being proportional to their surface activity. The phosphoric acid-choline polar group of lecithin and the phosphoric acid-ethanolamine polar group of cephalin would orient themselves toward the aqueous phase. They would become solvated in pure water, but, in the presence of plasma proteins, functional groups of both phospholipids and proteins likely would react through primary valences to form salt-like compounds. It is known that lecithin and cephalin form complexes with bacterial cells which inhibit the action of synthetic detergents (1). Cephalin has been shown to react with serum albumin and salmine, the rate of reaction being high; lecithin appears to react more slowly (2). Other

less stable complexes are possible through secondary valences or adsorption. Macheboeuf and Sandor (13) voiced similar ideas in connection with blood.

This speculation supports the theory advanced by Rimpila and Palmer (20), *viz.*, "It appears possible that the 'membrane' may be formed before the fat globules become a part of the milk or that the fat globules may be secreted before the milk plasma is completely formed, in which case the 'membrane' materials could be considered, in part at least, as precursors of plasma materials." The speculation is not counter to the idea of a special 'membrane' protein, or to the finding that the membrane protein of cream washed four times with water, four times with rennet whey, and then four times with water, and that of an artificial whey cream washed three times with water, have a sulfur content practically identical with the natural membrane. The membrane itself was lower in lipid phosphorus when whey was not used (20). The speculation is in keeping with the lipid extraction data of Tayeau (26), who found 20 per cent of the phospholipids in milk extractable with ether, 30 per cent with ether and soap, and the remainder extractable by ether after treatment with boiling alcohol to denature the protein in combination with the phospholipids.

As an outgrowth of these studies the authors have adopted the practice of incorporating phospholipids in the fat as well as in the whey, skim milk, or other media in making remade milks or creams, providing the media do not already contain appreciable phospholipid or the lipoprotein complex; buttermilk requires none. The principle has found industrial application in making wartime substitutes for ice cream and also genuine ice cream (28). Lecithin, for example, is separately incorporated into both the aqueous and fatty phases. Josephson and Dahle (10) succeeded in imparting normal whipping properties to ice cream mixes made with butter or butter oil as the source of fat by emulsifying the fat with either dried egg yolk or the natural "membrane suspension" before incorporating the fat into the mixes. These authors considered that a protein-phospholipid complex, already formed, was essential for proper emulsification; specificity was allotted to the true "membrane" protein moiety of the complex. Sell *et al.* (23), in studies with mayonnaise, showed that the lecitho-protein complex of egg yolk was the emulsifying agent in egg yolk, whereas free lecithin and free cephalin were detrimental. The results of the present study would seem to indicate that unaltered serum proteins with functional groups available for combining with functional groups of the phospholipids are adequate for the formation of such complexes.

SUMMARY

The role of milk fat in the foaming of milk, cream, buttermilk, and their artificial counterparts has been studied at temperatures between 5 and 50° C. The results suggest the following conclusions.

1. In such emulsions two types of foam may appear separately or simultaneously, a protein type and a phospholipid-protein type. At the higher temperatures the protein type predominates.

2. Whole milk, cream, and buttermilk exhibit minimum foaming at 30-35° C.

3. At 35° C. the foam volume and the foam stability of skim milk are decreased as the fat content is increased up to about 5.0 per cent. With further increases in the fat content both the volume and stability of the foam increase up to a fat content of 20 per cent, after which no further increases occur. At 6° C. the foam volume remains constant regardless of the fat content. The stability of the foam reaches a minimum at about 5 per cent fat concentration, after which it increases rapidly until a fat content of 10 per cent is reached, above which cream-type foams of high stability are formed.

4. Artificial milks and creams were made to resemble the natural product only when phospholipids (soybean) were incorporated into the fat prior to emulsification. The medium should be a protein sol; a lactalbumin sol or a milk serum protein sol such as rennet whey was satisfactory. The most normal cream was made when the medium contained natural fat globule "membrane" material; buttermilk met this condition.

5. Emulsions of pure milk fat in skim milk, gelatin, or gum arabic sols have abnormal foaming properties.

6. For emulsions with fat dispersed to a degree comparable to that of natural milk or cream, the optimum concentration of mixed phospholipids in the fat appears to be from 0.8 to 1.0 per cent. Unbound phospholipid appears undesirable.

7. These results have been discussed as they apply to churning, to cream whipping, to cream rebodding, and, by inference, to ice cream whipping.

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A STUDY OF MULTIPLE BIRTHS IN A HOLSTEIN-FRIESIAN HERD¹

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Dairy cows are uniparous animals. Multiple births are relatively rare and occur with variable frequency. Twin births are most frequent, and triplets, quadruplets, and quintuplets are progressively rarer. Numbers of twin births reported for individual dairy breeds range from less than 0.5 per cent to 4.5 per cent (11) and for individual herds up to 8.8 per cent (9).

There still are differences of opinion as to whether twin births are desirable or undesirable in dairy cattle breeding (4, 5). Hewitt (7) considers multiple births a sign of increased fecundity and fertility, whereas Williams (12) relates such births to unsound or even diseased conditions of the genital tract, in particular of the ovary.

A general study of fertility in dairy cows, in which twin births were recognized as one of the factors influencing reproductive performance, led to a more detailed investigation of multiple births. The results are presented herewith.

SOURCE OF DATA

The data for this study were taken from the records of the Holstein-Friesian experimental herd at the New Jersey Agricultural Experiment Station and cover a period of about 15 years. The breeding program and operations pursued in this herd are rather unique insofar as the herd is self-containing and inbreeding is practiced to a high degree. The inbreeding with rigid selection is manifested in the preservation and concentration of the young animals' relationship to the noted sire, Ormsby Sensation 45th. In publications of Bartlett and Margolin (1) and Bartlett *et al.* (2, 3) detailed accounts are given of the conduct and progress of this experimental breeding project. From these reports it is evident that the artificial selection was not specifically directed toward reproductive efficiency and twinning but toward such qualities as milk and butterfat production, butterfat percentage, and body conformation.

RESULTS

By nature of the character investigated, the results must be so evaluated

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that small changes in actual numbers could have notable effect upon some of the summaries and averages.

General occurrence of multiple births. From 1931 to 1946 there were 937 parturitions and abortions available for observation. Of these, 37 or 3.95 per cent were twin births and 2 or 0.21 per cent triplets, making a total of 39 or 4.16 per cent multiple births. In other words, the average incidence of twin births was one in every 25.3 and triplets one in every 468.5 births, or one multiple birth in every 24.0 births. The 39 multiple births were observed in 36 cows; three cows gave birth to twins twice. Since only two sets of triplets occurred and the gestation of one set ter-

TABLE 1
Multiple births in relation to age of dam

Age by parturition no.	Observed parturitions	Observed multiple births	
	(no.)	(no.)	(%)
1	269	2	0.74
2	199	10	5.03
3	142	6	4.23
4	100	5	5.00
5	77	6	7.79
6	51	5	9.80
7	41	3	7.32
8	30	0	0
9	23	1	4.35
10	4	1	25.00
11	1	0	0
Summary	937	39	4.16

minated in an abortion without sex determination of the fetuses, it was considered advisable in these investigations to include the triplets along with the twin births.

Twin births in relation to age of dam. Frequency of twinning in relation to age of dam is summarized in table 1, where age is expressed in parturition numbers. The first parturition occurred at an average age of about 29.5 months, and the average interval between parturitions was about 14.5 months. This tabulation shows that the number of twin births has been extremely low at the first parturition. From then on it increased with age at calving, first increasing abruptly at the second parturition and then gradually reaching a peak at the fifth, sixth, and seventh par-

turitions. Above these ages a decrease seemed to take place, but in view of the small number of cases not too much weight should be given to this observation.

Length of twin gestations. Confirming Hewitt's findings in British Friesian cows (7), twin gestations in this herd were of shorter duration than gestations of single calves. The highly significant difference amounted to about 7 days. Between the sexually unlike twin pairs, differences also existed, as shown in table 2. The mean length of gestation of the male pairs was greater than that of the sexually-mixed pairs and considerably greater than that of the female pairs. It appears, as in the case of single calves (10), that sex of the fetuses has an influence upon length of gestation.

Sex ratio. The sex ratio of 36 twin and one triplet births was found to be 33 males to 42 females, or 44 per cent males. The theoretical ratio

TABLE 2
Length of gestation of twins

	No. of gestations observed	Length of gestation	
		Mean days	Standard deviation days
Male pairs	6	275.33 \pm 3.97	6.87 \pm 1.98
Sexually-mixed pairs	11	273.64 \pm 2.74	8.23 \pm 1.75
Female pairs	14	270.00 \pm 2.56	8.86 \pm 1.68
Summary	31	272.99 \pm 1.68	9.37 \pm 1.19

of twin pairs, 1 ♂♂ : 2 ♂♀ : 1 ♀♀ was met by an actual one of 9 ♂♂ : 13 ♂♀ : 14 ♀♀, or 1.00 : 1.44 : 1.56. Although these ratios are rather unusual, their differences from the theoretical are not statistically significant.

Vitality of multiple calves. The vitality of multiple calves was evaluated according to the number born dead or that died within 2 days after birth and by following up the individual life histories of female twin pairs.

The mortality rate at birth for each sex is presented in table 3. It was slightly higher for male calves than for females. The average for both was 22.67 per cent, in contrast with only 9.65 per cent for the whole herd.

The history of the 14 female twin pairs revealed that 6 individuals, or 21.43 per cent, were born dead or died soon after birth; 5, or 17.86 per cent, were sold when immature, mostly for reasons of selection; 6, or 21.43 per cent, freshened in the herd but showed relatively poor production and breeding records; only 3, or 10.72 per cent, remained in the herd

for the length of their natural life, averaging 8.5 years, and exhibited relatively good production and breeding records; and 8, or 28.57 per cent, still in the herd at the time of this writing were immature, ranging in age from 4 to 18 months.

The mortality rate and life history of these heifer calves strongly indicate that twins have a lower vitality and poorer prospects of productive life than single calves.

Retained placentae and twin births. The condition of retained placentae after twin births was very much aggravated when compared with births of single calves. Of 31 apparently normal multiple parturitions, 23, or 74.19 per cent, were accompanied by this condition. The percentage for the whole herd was 23.10.

Conception rate of dams after twin births. After giving birth to twins, the dam's conception rate for a succeeding pregnancy should be another

TABLE 3
Mortality of multiple calves

	Males	Females	Total
No. of calves	33	42	75
Dead and aborted calves	8	9	17
Per cent mortality	24.24	21.43	22.67

indication of the possible effect of twinning on future breeding efficiency. An analysis of the records revealed that 71 services were required for 22 safe pregnancies in as many cows after twin births, corresponding with a conception rate of 3.23. This conception efficiency was almost 50 per cent lower than that of the herd average, which amounted to 2.21.

Calving interval after twin births. After the 37 twin births, apparently normal pregnancies and parturitions were noted in 13 instances. The mean calving interval was 483.5 ± 22.3 days. This interval corresponded with a breeding efficiency of 75.41 per cent, which was considerably below the herd average of 82.59 per cent. This difference alone suggests that twin births cause a reduction in reproductive efficiency.

Influence of twin birth upon future reproductive performance. In the 39 cases of multiple parturitions, 15 cows, or 38.46 per cent, continued to produce in apparently normal fashion, except for the higher conception rate and longer calving intervals. On the average, these cows survived their twin calving age by 2.33 parturitions. Five cows, or 12.85 per cent, became sterile; 12 cows, or 30.77 per cent, were sold for various reasons shortly after giving birth to twins; and three cows, or 7.69 per cent, died after the twin parturitions, one because of hardware, another,

from a ruptured uterus, and the third from an unknown cause. Of the four remaining cows which have given birth to twins within the last 9 months and which are still in the herd, two already have exhibited breeding troubles.

This recorded information does not warrant definite conclusions in regard to the influence of twin births on the future reproductive performance of the cows. The five proved cases of sterility are not in excess of the expectation for the whole population. It must be remembered that the disposals include a number of cases which must be regarded as doubtful in this respect. On the other hand, the first group, which consists of the 15 cows with an apparently normal reproductive performance after twin births, comprises the largest proportion of the grouped twin dams. Their performance at least implies that twin births do not necessarily cause breeding troubles with sterility implications or shorten the reproductive life of the cows.

Breeding efficiency of twin dams. Previously it was shown clearly that twin births exert a depressive effect upon the dam's subsequent

TABLE 4

Breeding efficiency of twin dams compared with the herd as a whole

Group	No. of cases	Percentage of breeding efficiency			
		Mean	Standard deviation	Coefficient of variation	Skewness of distribution
Herd as a whole	144	83.99±0.94	11.32±0.66	13.48	+0.0483
Twin dams	26	83.46±1.59	7.96±1.10	9.54	—0.0302

breeding efficiency. That this will affect a cow's lifetime breeding efficiency in proportion to her life span is acknowledged for the following analysis.

The lifetime breeding efficiency was determined for 26 twin dams, the only ones with complete records available. Their mean breeding efficiency and the standard deviation and coefficient of variation, as well as the approximate measure of skewness, were compared with the respective values of the herd as a whole.

Table 4 shows that almost no difference existed in the mean breeding efficiency between these two groups. The values for the standard deviation and coefficient of variation were considerably smaller for the twin dams than for the whole herd, denoting a greater uniformity of the twin group. Moreover, the distribution of the twin group was skewed positively, that is, toward the higher values, while the distribution of the whole herd was skewed negatively. Since twinning in itself has a depressing effect

upon the breeding efficiency, as already established, and since the frequency distribution of the herd as a whole markedly is skewed toward the lower values, a strong argument is offered for a fundamentally higher breeding efficiency in favor of the twinning group.

Milk production of twin dams. A study of the relationship between twinning and milk production was another object of this investigation. For this purpose the records of the 26 twin dams already employed in the analysis for breeding efficiency were considered suitable for a comparison of the milk production between twin dams and the herd as a whole.

The milk yield in both groups was expressed in pounds of 4 per cent fat corrected milk (5) per day on a mature equivalent twice-a-day milking basis. The actual milk yield was converted to the mature equivalent by the use of conversion factors based on the production records of the herd itself. The daily average was calculated on the total adult days the individual cow stayed in the herd, starting at the age of 27 months and continuing until her last calving.

TABLE 5

Milk production of twin dams compared with the herd as a whole

Group	No. of cases	Milk yield in lb.			Skewness of distribution
		Mean	Standard deviation	Coefficient of variation	
Twin dams	26	30.08 \pm 1.06	5.32 \pm 0.74	17.70	— 0.7080
Herd as a whole	144	29.10 \pm 0.59	7.11 \pm 0.42	24.42	— 0.3047

The results are presented in table 5. The constants chosen for this comparison were the same as those used for the analysis of breeding efficiency. The mean milk yield was insignificantly in favor of the twin dams ($P = 0.70$). The standard deviation and the coefficient of variation of the twin dams were considerably smaller than those of the herd, indicating greater uniformity for the first group. The frequency distributions of both groups were skewed negatively. This analysis indicated that the twin dams were at least equal in milk production to the herd as a whole. Superiority on this basis alone could not be demonstrated.

Comparison of various characteristics of fertility in twin dams and in the whole herd. Although the results of some investigations (7) indicate that twinning in dairy cows is associated with high reproductive qualities, this question is far from settled. Few data are available for correct evaluation of fertility itself, much less in relation to twinning. Thus, although twinning may be an expression of female fecundity in itself, the structural form of the female reproductive tract seems to obstruct the expression.

This conflict could be noted in the records and investigational results already cited and might interfere with most comparisons.

Besides breeding efficiency, the following criteria were used in the evaluation of reproductive performance: Live and dead calves born, twin births, abortions, and retained placentae. The comparison between twin dams and a representative group of cows producing only single calves is shown in table 6.

From this tabulation it will be seen that with the exception of the per cent of live calves, these measures of fertility all were in favor of the cows giving birth to single calves. That the greater percentage of living calves

TABLE 6
*Comparison of various measures of fertility
between twin dams and other herd representatives*

	Twin dams	Herd representatives
Total no. of cows observed	28	142
Av. coefficient of inbreeding per cow	0.09	0.09
Av. gestation number observed	3.73	3.12
Av. conception rate	2.53	2.10
Based on total no. of parturitions:		
% of live calves	100.00	83.35
% of dead calves	17.50	7.83
% of multiple births	23.33	0.0
% of abortions	9.17	6.57
% of retained placentae	30.83	18.69

for the twin dams' group was due almost exclusively to the twin births was revealed simply by adding the percentages of live and dead calves in both groups and subtracting the percentage of multiple births from the twin dams' group. Should allowance be made for the lack of vitality of twin calves, this slight superiority would vanish.

Another important point in this tabulation is the average observed gestation number, which is considerably higher for the twin dams. Since the gestation numbers stand in relation to the average age at calving of the cows, their averages indicate strongly that the twin dams were older than the cows with only single calves. Two factors might have contributed to this effect. Probably the main factor was the expression of twinning relatively late in life, making the twin dams a selected group in this respect. Many of the younger cows in the second group were potential twin dams. The other factor would be that twin dams actually were longer lived.

Twinning and inbreeding. When twinning was set in contrast with the degree of inbreeding of the cows in the herd, as illustrated in table 7, a non-uniform positive trend between these two characteristics was observed.

This feature does not mean that twinning was dependently related to the degree of inbreeding as such. It indicated rather well, however, that the factors for the twinning disposition were present in some of the foundation animals. By directing the breeding operations to the inbreeding of such animals, these factors became more concentrated in some cows and expressed themselves more often than in the foundation cows.

The hereditary aspect of twinning. Heredity control of multiple births in mammals has been proved amply in sheep, goats and other animals. In

TABLE 7
Comparison of inbreeding with twinning

Group	Coefficient of inbreeding	No. of cows	No. of parturitions	No. of twin births	% of twin births
1	0.00	55	116	2	1.72
2	0.01-0.04	92	291	10	3.44
3	0.05-0.09	21	67	0	0.0
4	0.10-0.14	36	87	4	4.60
5	0.15-0.19	29	73	7	9.59
6	0.20-0.24	9	16	1	6.25
7	0.25-0.29	10	22	1	4.55
8	0.30-0.39	3	8	1	12.50
Total		255	680	26	3.82

dairy cows this proof is attained only with difficulty, because its relatively rare appearance and its dependence upon the dam's age frequently hide the presence of this character. The small number of offspring in dairy cows and, possibly, environmental influences upon twinning contribute to the difficulties. Statistical investigation of the problem would necessitate large numbers of reliable and complete records such as those accumulated in herd book organizations. Unfortunately, these generally lack completeness, because only promising offspring are reported. An alternative, used in the present study, is the investigation of individuals and family groups in large herds where complete records are kept over long periods.

In table 8 the occurrence of twin births by parturition numbers is summarized for the members of 21 cow families which make up over 90 per cent of the present herd. Cows of ten of these families never had any

twins recorded. In the remaining 11 families the rate of twin parturitions ranged from 2.63 to 18.18 per cent.

In table 9 the twin parturitions of the daughters of 19 sires are presented. These daughters are, for the most part, the same cows represented in the cow families listed in table 8. In grouping these cows according to their sires, it was found that nine sires did not have any daughters with twin births. The remaining ten sires had one or more daughters which gave birth to twins. On the basis of all parturitions of the daughters of

TABLE 8
Occurrence of twins by cow families

Cow family no.	No. of parturitions	No. of twin pairs	% of twin pairs
3	20	0	0.0
15	17	1	5.88
20	18	0	0.0
37	23	1	4.35
61	66	6	9.09
64	22	4	18.18
66	48	2	4.17
68	20	0	0.0
69	21	1	4.76
75	14	0	0.0
78	20	1	5.00
80	26	0	0.0
82	34	2	5.88
91	7	0	0.0
92	38	1	2.63
93	24	1	4.17
95	17	0	0.0
96	14	2	14.29
97	15	0	0.0
100	13	0	0.0
103	25	0	0.0
Total	502	22	4.38

these individual sires, the ten daughter groups varied from 2.27 to 14.29 per cent in twin births.

This variation in both groups might be attributed to three sources, namely, pure chance, environment, and the genetic twinning disposition of sires and dams. The emerging combination of the daughters' germ plasma founded upon the physical basis of heredity was the determining principle of this disposition. Environmental factors such as feeding and management probably were of very minor influence. Hormonal therapy, though of considerable importance in twinning, as shown by Hammond and Bhattach-

arya (6), was of no consequence in this herd. With the generally low percentage of twin births, the expression of the disposition was very uncertain and the chance factor could not be discounted.

By means of genealogical diagrams of cow families that have had a relatively high number of twin births it can be demonstrated that the disposition for twinning is inherited.

Figure 1 is a genealogical diagram of cow family 61. It is arranged with the foundation cow on top, her female progeny following down the

TABLE 9
Occurrence of twins by sires' daughters

Sire no.	No. of daughters included	No. of parturitions	No. of twin pairs	% of twin pairs
A	6	20	0	0.0
B	4	10	0	0.0
C	13	44	1	2.27
D	4	10	0	0.0
E	19	62	3	4.84
F	8	41	1	2.44
H	3	8	1	12.50
I	18	68	3	4.41
L	6	21	0	0.0
N	15	41	2	4.88
O	27	77	7	9.09
R	4	5	0	0.0
T	32	80	2	2.50
U	18	35	2	5.71
V	7	10	0	0.0
W	5	11	0	0.0
X	6	14	2	14.29
D-1	3	8	0	0.0
F-1	3	4	0	0.0
Total	201	569	24	4.22

line, generation by generation. The individual offspring is designated by herd number. After the cow's number is given in parentheses the number of her twin parturitions, if any. For the younger offspring alive in the herd at this writing, the letter *P* for prospect was added. Animals that are twins themselves are so designated by numbers in bold-face type. Below the female's identification number is ascribed the sire's identification, generally by a letter or letter with number. Beyond that, separated by a dash, the percentage of twin births of the sire's daughters is given as far as it is known.

The figure illustrates how the transmission of the disposition for twin-

ning may work out in a relatively large cow family. Foundation cow 61, herself a twin, was sired by bull *C*, whose daughters born in the herd showed only 2.3 per cent twinning. Cow 61 had five daughters with reproduction records. They were sired by three different bulls, making three of them full sisters by sire *E*, whose daughters averaged 4.8 per cent twin births. Their records and especially those of the three full sisters suggest strongly a segregation in the Mendelian ratio. Two of the three full sisters, namely nos. 67 and 294, started progeny lines of their own. Neither of these lines exhibited any twinning. The third full sister, no. 256, produced one pair of twins in her second parturition. Unfortunately, she did not leave any progeny in the herd. Her early expression of the twinning character suggests, however, that she was highly predisposed to it.

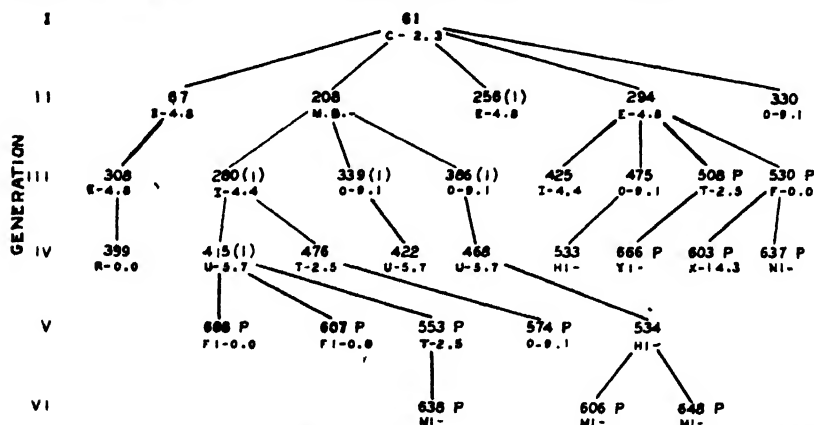


FIG. 1. Diagram of cow family 61 showing the extent and distribution of twin births, which amount to 9.09 per cent of all observed parturitions. For explanations of the diagram see text.

Daughter no. 208, which was sired by a bull designated *M. B.* and for which no twinning record could be established because of lack of daughters in the herd, started the longest line of progeny. Though no. 208 herself revealed no twinning disposition, her progeny visibly exhibited it to an extreme degree. Every one of her three daughters gave birth to one pair of twins. In turn, their progeny (generation IV) again suggest that Mendelian segregation might have been at work. The animals listed under generations V and VI are too young at present to allow any conclusion.

The fifth daughter, no. 330, of foundation cow 61 was sired by bull *O*, which was by progeny test a highly predisposed animal. Cow 330 calved only twice in the herd and produced single bull calves. Her disposition for twinning, therefore, never will be ascertained.

A similar investigation was made on cow family 64; her genealogy is diagrammed in figure 2. Family 64 was relatively small. Over a period covering five generations, this family was just about holding its own. In respect to twinning, this family's record, amounting to 18.18 per cent, was higher than that for any other cow family observed. The distribution of twin births extended over only three generations. The foundation cow did not visibly express any twinning disposition and the descendants in the fifth generation were too young to show any. A segregation into unusually predisposed and undisposed lines was hinted in this family, a feature closely resembling that of family 61.

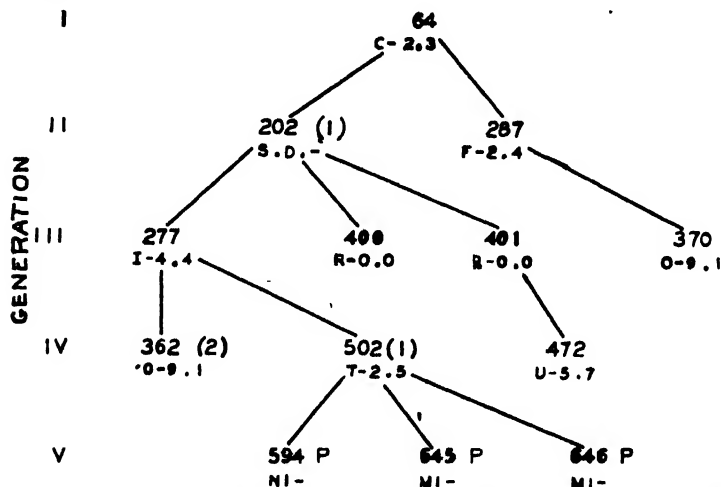


FIG. 2. Diagram of cow family 64 showing the extent and distribution of twin births, which amount to 18.18 per cent of all observed parturitions. For explanations of the diagram see text.

In both families virtually the same bulls appear as sires to the progeny. Sire *O*, with a high average twinning rate, is especially conspicuous as a sire of twin dams; so also is sire *I*, with a relatively low average rate. Both these sires are inbred to bull *C*, which is also the sire of both foundation cows. Sires *E* and *F*, both outbreds, are conspicuously present in the non-predisposed lines.

Obviously, the low frequency of twin births does not warrant definite conclusions with respect to the mode of transmission of twinning. The continued use of inbred sons and grandsons of bull *C* for several generations in succession and with it a concentration of certain genes of this bull suggest the possibility that he carried genes which foster twinning. In addition, it is observed that other inbred sons and grandsons of the same bull, as sires *T* and *R*, have very low rates for twinning. It should be

recalled that almost half of the cow families and half of the sires never showed any tendency for twinning. All of these observations point strongly to the conclusion that twinning in dairy cows is influenced chiefly by heredity yet expresses itself differently with age. The mode of transmission could well be understood by the assumption that twinning is under the control of a small number of autosomal genes which express themselves incompletely. The transmission seems to be recessive in character, with gene interactions or modifications.

DISCUSSION AND CONCLUSIONS

The observation made in this investigation that twinning is rare in the first parturition, rises to a peak in the fifth, sixth, and seventh parturitions, and then decreases with advancing age harmonizes closely with the general cycle of fecundity in most multiparous mammals. From this observation alone it is most probable that twinning is an expression of fecundity or the potential reproductive capacity of a dairy cow. However, if dairy cows are considered as strictly uniparous animals, and they should be so considered according to the structural development of their reproductive tract, twinning could be regarded as cases of reversion or atavism.

Evidence was presented that twinning is chiefly controlled by heredity. The effect of environment seems to be of very minor importance. The mode of transmission of the twinning character in dairy cattle is obscured by its relatively rare and incomplete expression, its sex-limited and age-limited appearance, and the small number of offspring inherent in cattle. The factual manifestations of transmission in two cow families over a number of generations provided impressive indications that twinning exhibits Mendelian segregation and seems to be under the control of a small number of genes. The character of twinning should be recessive with gene interactions or modifications. By making use of these findings it is feasible that in practical breeding operations twinning in dairy cattle could be influenced considerably in either direction.

The question then arises as to whether twinning is a desirable character in dairy cattle. If the available data all are accepted at their face value, twinning is associated with the increased production of calves by sheer numbers; if this fact is scrutinized from the actual reproducing value of the female line, the contrary is the case. Of the theoretical twin sex-ratio, 1 ♂♂ : 2 ♂♀ : 1 ♀♀, for all practical purposes only the female pairs are suitable for reproduction in the direct female lines. Theoretically, the number of these female twins would just be equal to the number of single females. Since twin calves have a higher mortality rate at birth and, apparently, a lower vitality throughout life than single calves, the real fact is that twinning has a harmful effect on the continuity of the female lines.

If the face value of data favoring the association of twinning with longevity is examined closely, the degree of association diminishes considerably. Twin births occur generally late in life, increase with advancing age, and are incomplete in appearance. If every animal in a group is expected to show this character, almost all the animals of this group have undergone an intense selection with respect to age. Therefore, almost any comparison of twinning in regard to age is of very questionable reliability.

Definite disadvantages of twin births include shortened gestation periods, greater parturition difficulties with subsequent increases in retained placentae, decreased conception rate, lower breeding efficiency, and increased sterility.

In summarizing all these factors, there is no doubt that twinning definitely is an undesirable character in dairy cattle, and efforts should be made to reduce its appearance by proper breeding methods and selection.

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A STUDY OF THE BROWNING REACTION IN WHOLE MILK POWDER AND ICE CREAM MIX POWDER¹

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The development of a brown color during storage is an index of deterioration in many foods. Browning during storage usually is not observed in commercial dry milk, but some observations on milk powders prepared for other purposes led to the consideration that incipient browning might be related to the development of stale and oxidized flavors in milk powder. Consequently, it seemed advisable to investigate the factors contributing to browning and the possible relationship to other deteriorative changes in milk powder.

REVIEW OF LITERATURE

The literature on the browning reaction in foods is voluminous. Excellent reviews of literature on darkening of various foods and fundamental aspects of the browning reaction have been presented in project reports, Committee on Food Research, QMC, in the last two years. The literature on browning reaction in dairy products has been reviewed recently by Sharp and Stewart (11). The browning in dairy products, similar to other foods, is attributed to two possible reactions: the caramelization of lactose and a Maillard-type reaction between lactose and milk proteins leading to formation of amino-sugar compounds. Webb (12) believes that a lactose-amino combination may account for much of the browning of autoclaved milk, with caramelization of lactose by phosphates as a contributing factor. Regardless of the mechanism of the reaction, the intensity of browning produced in milk is known to be influenced by certain factors, namely, the pH of milk (9, 13), lactose concentration (6, 9, 11), and temperature and time of heating (9, 13). The major chemical change in milk related to the browning reaction is a partial conversion of lactose into acids (7, 14). The acids produced are mainly lactic and formic (3, 7). Kometiani (7) could not account for the total increase in acidity in browning as derived from lactose. He attributed part of the increase in acidity to an increase in free carboxyl groups in the casein molecule.

The published information on browning of dry milk products is limited to the study of Doob *et al.* (2) on browning of dried whey and skim milk. Both products were roll-dried and none of the samples studied was gas-packed. The browning of these products was affected chiefly by moisture

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content, temperature, and time of storage. According to the authors, browning is markedly accelerated at temperatures above 30° C.; however, even at 50° C. browning could be inhibited by low moisture content. An increase in titratable acidity and a decrease in pH accompanied browning of dried whey and skim milk.

EXPERIMENTAL PROCEDURE

All powders of whole milk and ice cream mix used in this study were made by the spray process under standard commercial conditions of processing. Ice cream mix powder was prepared in a commercial plant with a Rogers-type drier. Whole milk powder was prepared in a commercial experimental plant equipped with Mojonnier stainless steel processing equipment and Mojonnier drier. All samples were packed and stored in 1-lb. tin cans unless otherwise specified. Gassing of samples refers to evacuation and gassing with nitrogen. Gas analysis was made according to procedure of Peters and Van Slyke (8) with a Haldane gas apparatus. The pressure of gas in cans was taken at 22–24° C. with an attached gas apparatus manometer built for this purpose. The degree of browning (except for the first experiment) was measured by visual comparison of the sample with a set of dry powder standards, the procedure developed by Doob *et al.* (2). These standards are made up of mixtures of potassium chromate, ferric oxide, Norrit and sodium chloride to give colors ranging from white to medium brown in 14 divisions and numbered from 0 for the white to 13 for the darkest standard. Ascorbic acid and total reducing substances were determined by indophenol titration, as described by Sharp (10) and modified by Doan and Josephson (1). In one experiment the reducing substances were determined also by a modified Chapman method and expressed in terms of ferricyanide values. Moisture was determined by the toluol-distillation method, and the solubility index by the method recommended by the American Dry Milk Institute. The QMC score card of 1 to 15 was used in assigning the numerical value of the organoleptic score.

EXPERIMENTAL RESULTS

Experiment I. Effect of high humidity on browning of milk powder. In this experiment, samples of freshly prepared whole-milk powder were vacuum-packed in special laminated packages, shown by previous experiments to be water-vapor permeable but supposedly not air permeable.² The vacuum-packed samples were placed in a wet incubator at 95 per cent relative humidity and the thermostat set at 40° C. During the experiment the thermostat stuck and the temperature rose to 45° C. or more. This

² The authors are indebted to Mr. W. C. Cole and Mr. E. S. Chase, Research Laboratory, Arden Farms, Los Angeles, for their valuable contribution in securing the data of Experiments I and II.

increase in temperature undoubtedly accelerated the changes under consideration but did not destroy the value of the experiment. Samples were withdrawn at intervals from the incubator for testing. Results of these tests are presented in table 1.

Storage of whole milk powder under high relative humidity at 40 to 45° C. led to a rapid browning of samples. The browning was accompanied by a decrease in vitamin C, decrease in solubility, and increase in ferri-cyanide value. With the increase of moisture in powder, there was a development of mild stale flavor at first; but as browning appeared and developed, the stale flavor either decreased or was masked by a burned or caramelized flavor.

Experiment II. The effect of moisture content of powder on the rate of browning. The effect of moisture content of powder on the rate of browning also is brought out by the data of Experiment II. In this experiment, samples were prepared to contain approximately 4 per cent and 7 per cent moisture by adding water drop by drop from a pipette to milk powder as it was stirred in a Hamilton Beach Mixer. The prepared samples were packed: (a) in air, using pint mayonnaise jars, and (b) vacuum-packed (vacuum of 28 inches) in special vacuum-holding laminated packages. Samples were stored in an incubator at 45° C. Subsequent analyses showed that within the same moisture group of samples, there was a variation in the moisture up to 0.9 per cent. The vacuum-packed samples lost some moisture as the result of subjecting the samples to vacuum treatment. The data on browning and reducing groups (ferri-cyanide value) of the samples are presented in table 2. At 4 per cent moisture and storage at 45° C. there was no significant change in color on storage for 26 days. Samples containing 7 per cent moisture darkened significantly within the first 2 weeks of storage. Air-packed powder showed no consistent and significant difference in the degree of browning as compared with the same powder vacuum-packed at the levels of moisture tested.

The solubility index was run on the samples after 2 and 3 days of incubation. By then, the high-moisture samples were very insoluble, and continuation of the tests seemed unnecessary.

Experiment III. Gas changes during browning of whole milk powder. Early observations on browning of some ice cream mix powder samples packed under air and under 3 per cent of oxygen indicated that the browning was retarded in gas-packed samples. In one case, ungassed ice cream mix powder on storage at 45° C. for 3 months was dark brown and the same product gassed was only very slightly discolored. These samples originally were below 2 per cent in moisture but were badly contaminated with iron, which might explain why browning took place at that level of moisture in powder. Other samples of powder, free from iron contamination and with

TABLE 1
Effect of storage under high relative humidity on browning of milk powder

Length of storage at 40-45° C.	Moisture	Flavor	Color	Solubility index	Texture	Reducing substances		
						Vit. C	Dye titration	Perisyanide value
(Ar.)	(%)					(mg./l.)	(mg./l.)	
Control	2.25	11.5	Cream	1.20	Fluffy	13.3	12.4	10
17	3.32	11.5	Cream	1.45	Caked edges	12.9	14.3	11
24	3.73	10.5	Cream	1.55	Caked edges	13.4	14.7	11.5
41	4.61	10.5	Cream	2.00	Caked edges	12.2	12.6	12.5
48	4.97	10.5	Cream	1.85	Caked edges	11.6	12.6	13
65	5.59	9.0	Sl. brown	1.90	Hard	12.3	11.7	15
113	5.86	7.0	Brownish	7.00	Hard	9.5	11.0	25
161	7.49	6.0	Brown	7.90	Hard	7.4	11.2	33
353	7.68		V. brown	7.90	Hard	4.7	35.0	> 80

TABLE 2
Effect of moisture and atmospheric pressure on browning of milk powder

Incubation time at 45° C.	Color				Ferryanide value				Solubility index		
(days)	4A ^a	4V	7A	7V	4A	4V	7A	7V	4A	4V	7A
2	1.0	0.9	1.5	0.9	18	19	28	25	0.60	0.65	8.25
3	1.0	0.9	1.5	0.9	14	14	25	25	0.95	0.90	9.20
6	1.0	0.9	1.5	1.0	15	15	34	35			
15	1.0		2.25		30		60				
26	1.0		3.25		40		55				
33	1.75		3.75	3.75	50		70	100			
61	2.75	3.0	4.50		100	150	100				

^a 4A — air-packed—4% moisture.
 4V — vacuum-packed—4% moisture.
 7A — air-packed—7% moisture.
 7V — vacuum-packed—7% moisture.

moisture content of 2.5 per cent or lower, did not show any discoloration on storage at 44–45° C. for over 1 year. It is probable that iron accelerates browning in milk powder, as it has been shown to do in orange juice (5) and in lemonade and orangeade powders (4). This acceleration of browning does not explain the difference, however, in degree of browning in gassed and ungassed samples, unless removal of oxygen inhibits browning as in the case of orange juice (5). The data of Experiment II in this report show clearly that in samples of powder of high moisture content the degree of browning was not affected significantly by a partial removal of oxygen.

In Experiment III the samples of whole-milk powder were prepared to contain approximately 7, 4, and 2 per cent of moisture. This was accomplished by placing freshly prepared powder at 2 per cent moisture in a special stainless steel chamber of high relative humidity. The powder in this chamber was mixed frequently, and incorporation of moisture up to 7 per cent was accomplished in 44 hours. The samples at each level of moisture content were packed under three levels of oxygen, that of 21 per cent, about 10 per cent, and less than 2 per cent by packing, respectively, in air, with single gassing and double gassing. All samples were packed to contain 14 oz. of powder. The free-space gas volume, as calculated from gas pressures at 23° C., was 495 ± 10 ml.

All samples were stored at 40° C. After 5 months of storage, the samples of 2 per cent and 4 per cent moisture failed to show a significant discoloration at 40° C. and were placed in the incubator at 60° C. for further storage. The data on browning, uptake of oxygen, production of carbon dioxide, and changes in moisture and flavor are presented in tables 3, 4, and 5 for powders of 7, 4 and 2 per cent, numbered as series 29II, 29I and 29, respectively. The partial pressure values for carbon dioxide and oxygen were calculated by converting per cent of gas on wet basis to per cent on a dry basis and multiplying this figure by total pressure on a dry basis.

Figures 1 and 2 show the rate of browning and gas changes during browning of high-moisture powder. The relationship between degree of browning and carbon dioxide production in powder of 2 per cent moisture packed under various levels of oxygen is shown in figure 3.

The data on browning and other changes of ice cream mix powder stored at 20, 37 and 45° C. are given in table 6. This powder had no added sugar and contained 53.55 per cent fat. The powder had 0.04 per cent tannic acid added as an antioxidant during the processing of the mix.

DISCUSSION

The conditions under which dry milk and ice cream mix powder undergo darkening or browning as a result of aging are apparent from the data presented. The browning of powder is a function of its moisture content

TABLE 3
Browning of whole milk powder of 7% moisture packed under various levels of oxygen (storage at 40° C.)

Oxygen level (%)	Sample	Storage time (days)	Moisture (%)	Color	Partial pressure			Flavor
					CO ₂	O ₂		
21	1	0	7.00	0.9	(mm.)	(mm.)		
	2	6	7.04	1.5	5.0	146.4		Good
	3	28	7.04	2.6	21.3	103.4		Burnt & stale
	4	47	7.00	4.0	39.7	1.3		Burnt & stale
	5	70	6.90	4.7	49.4	0.3		Caramelized & stale
	6	104	7.36	6.7	61.2	0.3		Caramelized & stale
10	1	0	7.22	0.9	3.5	59.0		Good
	2	6	7.34	1.7	11.1	32.4		Burnt & stale
	3	28	7.20	3.8	28.7	1.4		Caramelized & stale
	4	47	7.40	4.3				Caramelized & stale
	5	70	7.52	6.9	48.5	0.2		Caramelized & stale
	6	104	7.30	7.1	53.6	0.2		Caramelized & stale
2	1	0	6.74	1.0	1.1	2.8		Good
	2	6	6.70	1.8	6.1	1.0		Burnt
	3	28	7.00	5.3	22.7	0.8		Caramelized & v. sl. stale
	4	47	6.80	4.8				Caramelized & v. sl. stale
	5	70	7.14	7.5	42.7	0.7		Caramelized & v. sl. stale
	6	104	7.20	8.0	54.7	0.2		Caramelized & v. sl. stale

TABLE 4
Browning of whole milk powder of 4% moisture packed under various levels of oxygen

Powder	Sample no.	Storage time (days)	Storage temperature (° C.)	Moisture (%)	Color	Partial pressure		Flavor
						CO ₂	O ₂	
29I	1	0		3.93		(mm.)	(mm.)	
	2	19	40	3.73	0.7	2.0	156.8	Good
	3	61	40	3.62	0.9	4.7	142.8	
	4	152	40		1.0	13.2	84.6	
	5	11	60	5.25	< 1.5			Stale
	6	15	60	5.29	< 13.0	215.0	< 1.0	V. caramelized
29IA	1	0		3.70		> 230.0		
	2	19	40	4.04	0.7	1.5	91.5	Good
	3	61	40	4.00	0.9	3.2	86.5	
	4	152	40		1.0	13.1	35.7	
	5	11	60	5.56	< 1.5			Stale
	6	17	60	5.56	> 13.0	277.0	< 1.0	V. caramelized
29IAB	1	0		3.87		> 250.0		
	2	19	40	3.96	0.7	1.1	6.6	Good
	3	61	40	3.80	0.9	2.3	5.9	
	4	152	40		0.9	3.6	2.5	
	5	11	60	4.94	< 1.5			Sl. stale
	6	17	60	4.80	> 13.0	119.0	< 1.0	V. caramelized
						> 260.0	0.0	

TABLE 5
Browning of whole milk powder of 2% moisture packed under various levels of oxygen

Powder	Sample no.	Storage time (days)	Storage temperature (° C.)	Moisture (%)	Color	Partial pressure		Flavor
						CO ₂	O ₂	
29	1	0						
	2	152	40	1.95	0.6	2.8	141.7	Good
	3	17	60	2.30	0.6			Stale
	4	43	60	2.00	1.5	16.6	<1.0	Stale
	5	108	60	2.06	1.8	23.4	<1.0	Stale & caramelized
	6	157	60	2.20	4.2	80.2	<1.0	Stale & caramelized
29A	1	0						
	2	152	40	1.82	0.6	2.1	88.2	Good
	3	17	60	1.70	0.6			Stale
	4	43	60	1.80	1.5	16.2	<1.0	Stale
	5	85	60	2.10	1.8	20.8	<1.0	Stale & caramelized
	6	108	60	2.40	4.5	72.6	<1.0	Stale & caramelized
	7	157	60	2.90	3.8	55.2	<1.0	St. stale & caramelized
29AB	1	0						
	2	152	40	1.72	0.6	1.6	3.5	Good
	3	17	60	1.40	0.6			Good
	4	43	60	2.00	1.5	11.5	<1.0	Good
	5	85	60	1.34	1.8	15.1	<1.0	Good
	6	108	60	1.80	2.0	21.1		
	7	157	60	2.32	2.5	27.7		St. caramelized
					3.5	42.4		

TABLE 6
Browning of ice cream mix powder

Sample no.	Packaging	Storage temperature (° C.)	Storage time (days)	Moisture (%)	Color	CO ₂ (%)	O ₂ (%)	Titratable acidity (ml. 0.1 N NaOH)	Flavor
1	No gassing	20	26	1.5	1.5	0.12	20.7	1.36	Good
2	No gassing	20	80	1.5	1.5	0.18	19.77	1.36	Stale
3	No gassing	20	431	1.60	1.5	0.50	13.70	1.36	Good
4	Gassed	20	26	1.50	1.5	0.03	2.28	1.49	Good
5	Gassed	20	80	1.5	1.5	0.08	1.49	1.36	Good
6	No gassing	37	0	1.5	1.5	0.12	20.70	1.40	V. stale
7	No gassing	37	416	1.96	1.5	1.49	2.40	1.36	Good
8	Gassed	37	0	1.50	1.5	0.03	2.28	1.36	V. sl. stale
9	Gassed	37	416	1.60	1.7	0.31	0.03	1.36	Good
10	No gassing	45	0	1.50	1.5	0.12	20.70	1.36	Good
11	No gassing	45	14	1.60	1.5	0.39	19.02	2.54	Oxidized & caramelized
12	No gassing	45	55	1.5	1.5	0.91	13.49	1.36	Good
13	No gassing	45	416	1.95	4.0	0.03	2.28	2.20	Sl. stale, sl. caramelized
14	Gassed	45	0	1.50	1.5	0.44	0.84	5.10	Caramelized & stale
15	Gassed	45	55	1.5	1.5	1.28	0.06	4.60	Caramelized
16	Gassed	45	416	1.70	2.7				
17 ^a	No gassing	60	40	2.20	10.0				
18 ^a	Gassed	60	40	1.90	7.0				

^a Samples 17 and 18 are duplicates of Samples 7 and 9, respectively. They were stored at 37° C. for 416 days and then placed in an incubator at 60° C.

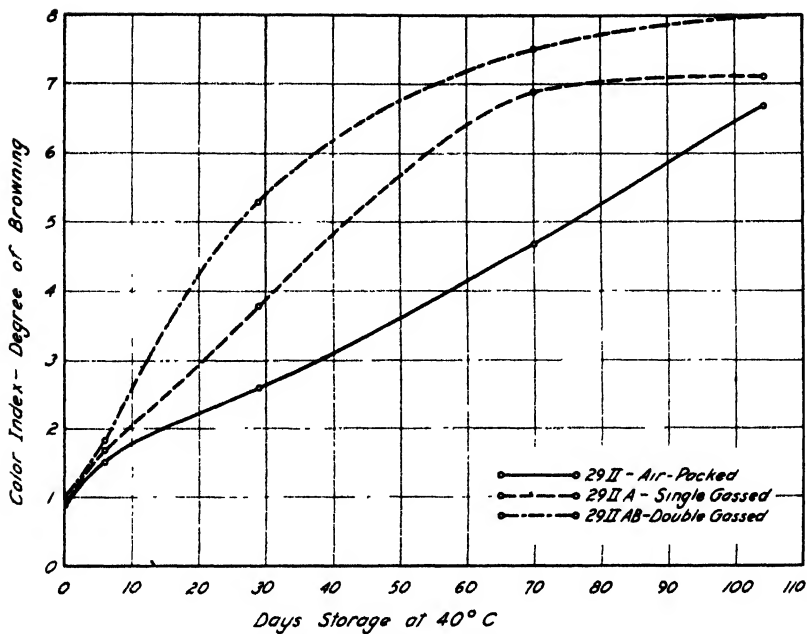


FIG. 1. Rate of browning of whole milk powder of 7% moisture (Series 2911).

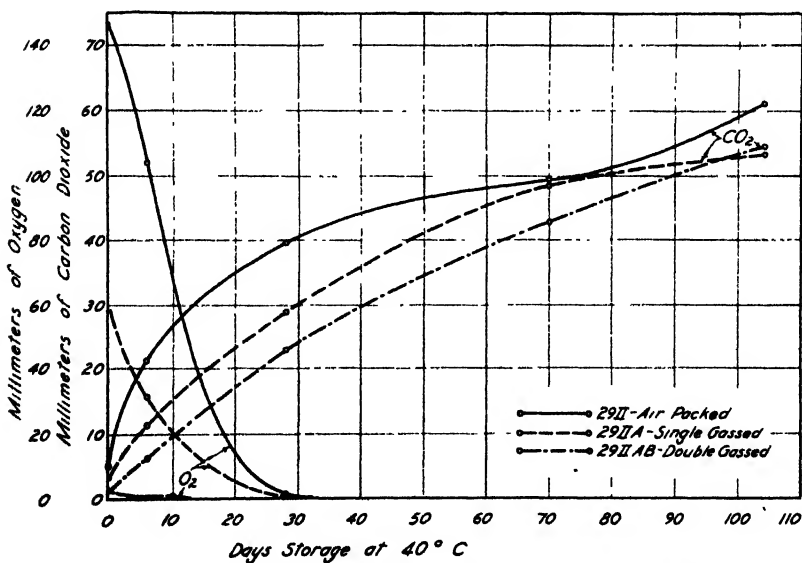


FIG. 2. Gas changes during browning of whole milk powder of 7% moisture (Series 2911).

and temperature of storage. Other factors, such as contamination with iron and possibly copper, certain added polyphenol compounds as antioxidants, and possibly vanillin in ice cream mix powder, may accelerate the rate of browning, but these factors are of comparatively minor importance in their relation to the browning of powder.

In general, the browning of powder is accompanied by: (a) production of carbon dioxide, (b) uptake of oxygen, (c) increase in reducing groups, (d) very marked decrease in solubility, (e) increase in titratable acidity,

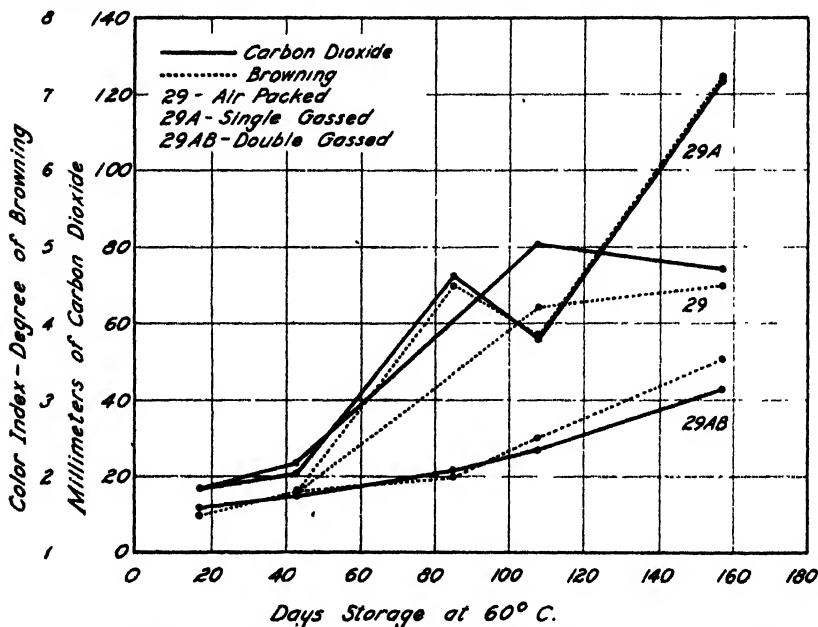


FIG. 3. Relationship between degree of browning and carbon dioxide production in whole milk powder of 2% moisture (Series 29).

and (f) development of caramelized flavor. In the advanced stages of browning there is also an increase in moisture content of powder. There was little, if any, increase in moisture in powder of 7 per cent moisture as compared to the same powder of lower moisture content.

In powder of 7 per cent moisture stored at 40° C., noticeable discoloration of powder takes place within a few days. On storage for a month, the powder becomes distinctly brown, with the appearance of caramelized flavor. At this time the oxygen practically is all gone and the partial pressure of carbon dioxide is increased to about 30 mm. under the conditions described. After the oxygen is gone, the browning and production of carbon dioxide continue but at slower rates.

In powders of 4 per cent and 7 per cent moisture content, storage at a

partial pressure of oxygen below 7 mm. (less than 1 per cent) did not retard the rate of browning. In fact, the samples packed under less than 2 per cent of oxygen have shown greater darkening than air-packed samples (see table 3 and fig. 1).

The browning of powder of 2 per cent moisture or less seemingly was retarded by packing the powder at the level of about 2 per cent of oxygen (see fig. 3 and table 6). It is possible that the retarding effect was due, at least partially, to a lower moisture content of gas-packed samples resulting from the vacuum treatment in the process of gassing.

It is evident from the data of tables 1, 4, 5 and 6 that both dry milk and ice cream mix powder of a moisture content below 4 per cent and stored at 40° C. or lower do not darken or brown in storage. Other samples of dry milk and ice cream mix powder of less than 3 per cent moisture have been stored at 40 and at 30° C. for over 2 years without showing any noticeable discoloration.

The usual deterioration in flavor of dry milk and ice cream mix powder in storage is independent of browning. Under the conditions of high available oxygen, as in the case of air-packed powder and storage at 40° C., the stale and oxidized flavor will develop with no browning at all. The same is true for a storage of powder at room temperature for a long period of time. The caramelized flavor is the only flavor that is produced by browning, and its intensity parallels the degree of browning. Caramelized flavor in gassed samples is a typical flavor of caramel. The development of stale or oxidized flavor apparently ceases when browning begins. There is no evidence that stale or oxidized flavors which have developed prior to browning disappear as browning progresses. These flavors merely are reduced or covered up when caramelized flavor appears.

SUMMARY

The conditions with respect to moisture content of powder, temperature of storage, and level of oxygen in gas-free space of container, as they may affect the browning of dry milk and ice cream mix powder as a result of aging, are given. The changes accompanying the browning, such as production of carbon dioxide, uptake of oxygen, increase in reducing groups, decrease in solubility and development of caramelized flavor, have been studied, and the extent of these changes in relation to the degree of browning is presented.

The darkening or browning of dry milk and ice cream mix powder, unlike some other dehydrated foods, is not related to the usual storage deterioration in flavor. The most common defective storage flavors of dry milk are the stale and the oxidized flavors. The development of these flavors in milk and ice cream mix powder is not the result of incipient browning.

In fact, it appears that the products of browning reaction inhibit the development of these flavors. Browning is accompanied by a development of a specific flavor, a caramelized flavor.

The browning does not take place in dry milk or ice cream mix powder when the above products are stored at 40° C. or lower if their moisture content is below 4 per cent.

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IRON AND COPPER CONTENT OF NON-MILK PRODUCTS COMMONLY USED IN ICE CREAM

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There is a lack of information on the iron and copper content of the non-dairy ingredients that commonly are used in conjunction with dairy products in the manufacture of ice cream. These materials include such products as stabilizers, emulsifying agents, sugar, cocoas, vanillas, chocolate liquors and coatings, and flavoring extracts. It is possible that one or more of these ingredients may contain enough copper or iron to accelerate the development of off-flavors of the oxidative type. Such metal contamination would be particularly important in the manufacture of dried ice cream mix that may be stored 6 to 12 months before using.

It was thought advisable, therefore, to study the iron and copper content of the previously mentioned materials. Accordingly, 74 samples of commercial non-dairy products commonly used in ice cream were analyzed. The iron content was determined by the method of Pyenson and Tracy (5) and the copper analyses were made by the method of Hetrick and Tracy (4).

EXPERIMENTAL RESULTS

The iron and copper content of stabilizers and sugars. Eighteen samples of stabilizers, gums, emulsifying agents, and sugars were analyzed for iron and copper. Magnesium nitrate (1) was added to Kragel,¹ sodium alginate, Irish moss, Vestirine and Gelox after carbonization to aid in the ashing. Two milliliters of a saturated solution of magnesium nitrate added after carbonization was found satisfactory to give a white, soluble ash. The results of the analyses are given in table 1.

All stabilizers except Gelox contained considerable amounts of iron. Irish moss contained 0.219 per cent of iron, which would be considered more than a trace amount. The emulsifying agents contained from 1 to 59 p.p.m. of iron. Egg yolk, which is sometimes used in ice cream as an emulsifying agent, contained 59 p.p.m. of iron. The sugars analyzed contained only small amounts of iron.

The copper content of the stabilizers varied from 0.92 to 10.0 p.p.m. Irish moss, locust bean gum, Kragel, and sodium alginate contained the most copper of the ten stabilizers analyzed. Glycerol monostearate did not contain any copper and Mixacoid contained practically none. Na-Pe-Co and egg yolk contained 2.85 and 3.35 p.p.m., respectively, of copper. Su-

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¹ Now modified and known as Kragelene.

crose, dextrose, and enzyme-converted corn sirup were found to contain less than 1 p.p.m.

The iron and copper content of American and Dutch process cocoas. The iron and copper contents of 17 samples of cocoa from five different manufacturers were determined (table 2). Of these samples, 13 had been treated with alkali (Dutch process) and four were untreated (American process).

The iron values for the American process cocoas varied from 73 to 119 p.p.m., with an average for the four cocoas of 92 p.p.m. In the Dutch

TABLE 1

The iron and copper content of some stabilizers, emulsifying agents and sugar.

Sample no.		Iron (p.p.m.)	Copper (p.p.m.)
	Stabilizers		
1	✓Gelatin (275 Bloom-pigakin)	14.8	2.21
2	✓Gelatin (125 Bloom-calfakin)	9.0	2.56
3	Gelox	1.2	2.0
4	Vestirine	28.0	1.75
5	Kragel	61.0	9.0
6	✓Sodium alginate	97.0	6.25
7	Irish moss	2190.0	10.0
8	Locust bean gum	16.4	9.0
9	Gum oat	47.6	5.4
10	✓Karaya gum	26.4	0.92
	Emulsifying agents		
11	Na-Pe-Co	19.4	2.85
12	Mixacoid	1.0	0.25
13	Glycerol monostearate	2.0	0.00
14	Egg yolk	59.0	3.35
	Sugars		
15	Sucrose, lot no. 1	0.9	0.15
16	Sucrose, lot no. 2	1.2	0.20
17	Dextrose	0.4	0.40
18	Enzyme-converted corn sirup	1.1	0.70

process cocoas the iron content averaged 117 p.p.m., with only three of them having an iron content under 100 p.p.m. Eight of the samples had an iron content between 110 and 149 p.p.m. There is some evidence that Dutch process cocoa contains more iron than American process cocoa, especially when comparisons are made between the two cocoas from the same manufacturer.

Dahlberg (2) found that a greenish-black discoloration of chocolate ice cream was caused by ferric tannate and that the cocoas that had a slightly alkaline pH value (Dutch process) were the only ones that produced the defect. The results of the present study suggest that the greater iron content of the Dutch processed product also may be a factor.

TABLE 2

The iron and copper content of American and Dutch process cocoas

Sample no.	Process	Iron	Copper
		(p.p.m.)	(p.p.m.)
	Brand A		
1	Dutch	94.5	20.6
2	American	73.0	21.0
3	Dutch	136.7	21.0
4	Dutch	142.7	21.2
5	American	84.7	20.6
6	Dutch	134.0	20.6
7	Dutch	134.0	21.0
	Brand B		
8	Dutch	149.0	22.2
9	American	119.0	23.4
10	Dutch	120.5	24.0
11	Dutch	141.0	21.6
	Brand C		
12	American	92.0	22.4
13	Dutch	110.0	23.5
14	Dutch	108.0	23.6
15	Dutch	105.0	31.2
	Brand D		
16	Dutch	69.0	27.3
	Brand E		
17	Dutch	75.0	28.3

The copper content of the samples varied from 20.6 to 31.2 p.p.m. American and Dutch process cocoas gave about the same copper values. The copper content of cocoas was more uniform than the iron content.

The iron and copper content of chocolate liquors and coating. Seven samples of chocolate liquor and one sample of milk coating were analyzed for copper and iron. The results are given in table 3.

Like cocoas, chocolate liquors and coating contain considerable quantities of iron and copper. The iron content of chocolate liquors and coating varied from 14 to 105 p.p.m., and the copper varied from 2.3 to 27 p.p.m.

TABLE 3

The iron and copper content of chocolate liquor and coating

Sample no.	Brand	Iron	Copper
		(p.p.m.)	(p.p.m.)
1	A	100.8	20.0
2	A (Milk coating)	14.0	2.3
3	B	59.0	14.9
4	B	71.0	15.5
5	C	104.5	27.0
6	D	103.0	24.8
7	E	105.0	18.9
8	F	19.4	9.8

The iron and copper content of flavoring. Twenty-two vanillas obtained from nine manufacturers were analyzed for iron and copper. The results are listed in table 4.

The three samples of powdered vanillas were uniformly low in iron and contained less than 2 p.p.m. copper. Powdered vanillas are made by grinding vanilla beans and combining the ground beans with a carrier like

TABLE 4
The iron and copper content of vanillas

Sample no.	Brand	Iron (p.p.m.)	Copper (p.p.m.)
Powdered vanillas			
1	A	4.8	1.35
2	A	4.0	1.90
3	B	1.4	0.45
Concentrated vanillas			
4	C	59.0	9.45
5	C	58.4	4.95
6	C	43.8	36.5
7	C	32.6	4.3
8	D	2.0	9.5
9	D	4.0	10.5
Vanilla extracts			
10	E	4.4	15.5
11	A	33.6	19.5
12	D	0.8	4.9
13	B	1.5	2.2
14	E	0.6	2.7
15	F	7.8	3.75
16	E	1.9	9.30
Imitation or partially imitation vanillas			
17	C	58.0	3.2
18	B	46.0	7.75
19	F	4.4	3.7
20	E	5.3	3.25
21	E	3.0	1.2
22	G	4.4	15.5

sucrose or glucose. The vanilla bean as it exists in nature appears to be relatively low in iron and copper.

The concentrated vanillas studied were found to have a rather high iron and copper content. The iron content of single strength vanilla extracts varied from 0.6 to 33.6 p.p.m. Five out of the seven samples had an iron content under 4.4 p.p.m. The copper content also varied considerably; the minimum was 2.2 p.p.m. and the maximum 19.5 p.p.m.

The iron content of the imitation or partially imitation vanillas varied from 3 to 58 p.p.m. and the copper content from 1.2 to 15.5 p.p.m. Represented in this lot were six samples from five different manufacturers.

The iron and copper content of fruit flavors and extracts. Most of the samples of fruit flavors and extracts analyzed (table 5) contained less than 5 p.p.m. of iron. Six out of the nine samples contained less than 5 p.p.m. of copper. Lime flavor, black raspberry concentrate and strawberry flavor were comparatively high in copper content.

TABLE 5

The iron and copper content of some fruit flavors and extracts

Sample no.		Iron	Copper
		(p.p.m.)	(p.p.m.)
1	Brand A		
	Orange emulsion conc.	3.6	0.25
2	Lemon emulsion conc.	3.4	0.25
	Brand B		
3	Black raspberry natural flavor conc.	18.6	6.9
4	Imitation pineapple flavor	3.9	0.45
5	Strawberry flavor	5.6	5.05
6	Peach flavor conc.	1.3	2.25
7	Lime flavor	1.2	11.25
8	Pistachio imitation conc.	0.5	4.05
9	Imitation banana	1.0	0.65

DISCUSSION

The determination of copper by the direct carbamate method used in this study does not entirely eliminate the interference of nickel. Nickel exhibits maximum absorption at a wave length of 385 m μ , while copper exhibits maximum absorption at a wave length of 440 m μ . Hetrick and Tracy (4) state that when 5 γ of nickel are added to 5 γ of copper, the error is 10.6 γ . Studies in the wave length at which maximum absorption occurs of the materials reported in this paper indicate that there was little, if any, nickel present.

The non-dairy products individually would produce only insignificant increases in the iron and copper content of ice cream. The Irish moss sample studied would be an exception, as it contained over 0.2 per cent iron. While the copper and iron content of the milk-product ingredients ordinarily would have a major bearing on the iron and copper content of the finished ice cream, the total added by non-milk products could be of such quantity as to be an important factor in the development of oxidized flavors.

Observations by Dahle and Folkers (3) and Tracy *et al.* (7) have shown that ice creams containing small amounts of fruit such as strawberries and pineapple develop a stale and/or oxidized flavor sooner than does vanilla ice cream. These authors believe that the off-flavor is due to the presence of copper and the acid of the fruit. Dahle and Folkers (3) state

that if the amount of copper in the mix equaled 1.3 p.p.m., the off-flavor always developed. Tracy *et al.* (7) state that in order to prevent the development of a stale metallic flavor in strawberry ice cream, the elimination of copper contamination is necessary. Other fruits found to accelerate the reaction responsible for the off-flavor were oranges and lemons. The copper content of any of the non-dairy products studied conceivably could be a factor in accelerating the development of the oxidized flavor, especially if used in strawberry, pineapple, orange and lemon ice creams.

The vanilla sample no. 4 in table 4 has been shown to have anti-oxxygenic properties (6) although it contains relatively large amounts of iron and copper, indicating that a substance may be relatively high in iron and copper and still have antioxygenic properties.

CONCLUSIONS

Stabilizers, cocoas, chocolate liquors, sugars, vanillas and fruit extracts were found to contain iron and copper. The copper and iron present in some of these products is thought to be significant from the standpoint of possible cumulative effect in hastening fat oxidation and the development of off-flavor in ice cream.

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THE DEVELOPMENT OF FLAVOR IN AMERICAN CHEDDAR CHEESE MADE FROM PASTEURIZED MILK WITH *STREPTOCOCCUS FAECALIS* STARTER¹

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This report is the first of several papers dealing with the development of a higher flavor in pasteurized-milk American Cheddar cheese. Cheddar cheese is made by a process that gives a long period for controlled lactic acid fermentation during manufacture. Most investigators have found that commercial lactic starters, chiefly *Streptococcus lactis* and *Streptococcus cremoris*, affect acidity without otherwise greatly affecting curing. As these starters in active growing condition are very important in both cheese manufacture and curing, it is obvious that proper acidity is very important in curing cheese. With the exception of some inoculations of certain lactobacilli, there have been no bacteria found that have aided in the development of good Cheddar cheese flavor. Furthermore, added enzymes, particularly lipases and proteinases, have not given very promising results.

In 1941 Wilson *et al.* (8) compared curing temperatures of 40, 50, and 60° F. They found 40° F. to be best for cheese made from poor milk, but 50° F. was preferred for cheese made from pasteurized milk of good quality. The type of curing was rather uncertain at 60° F. About this same time Dahlberg and Marquardt (2) showed that cheese made from either raw or pasteurized milk of excellent sanitary quality and ripened in vacuum in tin cans uniformly failed to develop Cheddar flavor in a year at 40° F., whereas some Cheddar flavor developed in 4 months at 50° F. and in 2 months at 60° F. In this study cheese made from raw or pasteurized milk of low bacterial count developed flavor uniformly; hence, it is evident that the effect of pasteurization of milk in slowing the curing of cheese is due chiefly to destruction of bacteria rather than the milk enzymes, and that this effect may be overcome in part by higher curing temperatures. The evidence indicates that thermoduric bacteria are a factor in cheese curing or that the pasteurized milk was recontaminated.

Consideration of the problem indicated little chance of success by the usual procedure of isolating bacteria from cheese and using them in

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its manufacture, as so much of this work already has been done. Rather, one might consider the characteristics of the bacterium desired in cheese making and curing and then ascertain if such a bacterium exists. For example, the desired bacterium should be universal in milk, as all good milk can be made into good cheese without adding this culture. Probably the organism should survive pasteurization. It should produce lactic acid from lactose rapidly and also be able to use lactates or other compounds in cheese as a source of energy; grow well at temperatures of 50° F. or less, and at temperatures as high as 106° F.; not produce gas in large amounts, although it might need to produce some carbon dioxide, as Dorn and Dahlberg (3) have shown cheese made from excellent milk yielded limited amounts of almost pure carbon dioxide during curing; be non-proteolytic and not produce objectionable flavors and odors. It should grow anaerobically at a pH of 5.0 to 5.5 and at salt concentrations up to 6 per cent, as this concentration is about the maximum that normally occurs in the water in Cheddar cheese. The bacterium which has been described obviously may be *Streptococcus faecalis*, as this organism has the characteristics given. This organism would develop in the cheese milk and in the cheese during curing, especially if stored at 50 or 60° F. White and Sherman (6) recently have found enterococci in all raw and pasteurized milk samples which they tested.

EXPERIMENTAL METHODS

A dozen or more cultures of *S. faecalis* were obtained from several laboratories and all of them produced acid too slowly in milk to appear to be promising. The idea remained dormant for a few years and then a search was made in nature for a strain which ferments lactose rapidly, as *S. faecalis* often loses this characteristic when propagated in media which do not contain lactose. Some 15 human adults saved stools from which enterococci were isolated on the penicillin-azide agar of White and Sherman (6), and *S. faecalis* was identified by the characteristics given by Sherman (5). *S. faecalis* is the predominating streptococcus in the digestive tract of man. Approximately 40 or 50 strains of enterococci were isolated before one was found that curdled milk rapidly. A 1 per cent inoculation of this strain incubated at 85° F. curdled milk in less than 18 hours, producing a smooth curd without gas. The flavor of the starter was characteristic and definite, but quite different from ordinary lactic starter. The odor was flat without being objectionable. It was identified as *S. faecalis* and possessed all typical characteristics. It did not ferment glycerol. The tyrosine decarboxylation activity of *S. faecalis* isolated from the starter was $Q_{CO_2} = 50$ and that isolated from the ripened cheese made with the starter was $Q_{CO_2} = 60$. This indicates a moderately active strain for conversion of tyrosine into tyramine.

This *S. faecalis* strain has been carried in milk pasteurized at 200° F. for 1 hour and incubated at 88° F. The curdled starters are held at 40° F. and transferred twice weekly. The starter appears to be pure *S. faecalis* on plating, but no endeavor was made to carry it in sterile milk or other media to assure no loss in acid-producing ability while the research was in progress. Should the starter become contaminated, the *S. faecalis* bacteria could be reisolated and developed as a new starter of the same organism. Pure cultures of this organism have been prepared and are in storage.

The milk used for cheesemaking was a good quality of market milk pasteurized at 143–145° F. for 30 minutes. After cooling to 86° F., the milk was divided into three lots of 300 lb. each. To the first batch of milk was added 2 per cent of Hansen's commercial lactic acid starter; to the second batch, 1 per cent of Hansen's starter and 1 per cent of *S. faecalis* starter; and to the third batch, 2 per cent of *S. faecalis* starter. The milk then was made into cheese according to the time schedule of Wilson (7), using the 4.5-hour schedule from adding rennet to milling the curd, except that no time was allowed for the starters to develop before adding rennet. Acid development was followed by titratable acidity and pH, using a Beckman pH meter, laboratory model G, with glass electrode. After manufacture and pressing, the cheese was vacuum packed in cans and ripened at 50 and 60° F. A few samples were made into 10- or 30-lb. cheese and paraffined in accordance with the usual commercial practice.

The cheese was analyzed for moisture, salt and fat. On the day it was taken from the press, analyses were made for pH, volatile acidity by the method of Kosikowsky and Dahlberg (4), and soluble nitrogen by the method of Sharp as reported by Dahlberg and Kosikowsky (1). The samples of cheese were scored by the authors at the end of one month curing and bimonthly thereafter. The samples were analyzed bimonthly for volatile acids, soluble nitrogen and pH.

A considerable number of series of cheese were made with remarkably consistent results, and two series made on different days are presented to illustrate the results.

RESULTS

The manufacturing data (table 1) show that the rate of acid development with the *S. faecalis* starter was slower than with the commercial lactic starter, and the mixture of the two starters developed acid at a rate intermediate between those of the two cultures used singly. *S. faecalis* grows well in the salt concentration of Cheddar cheese, so the pH of all samples of cheese 1 day old was rather uniform at pH 4.9 to 5.1 (table 2), irrespective of considerable variations in acidity present in the whey when

the curd was milled. The composition of the cheese was uniform, but the salt contents were somewhat low (table 1). Most other batches of cheese in other experiments contained 1.5 to 2.0 per cent salt.

While the cheese curd was matting in the vats, it generally was possible to observe that curd containing *S. faecalis* matted slightly more rapidly and that the curd developed more of the stringy character of the meat of chicken breast. As the time approached for salting, the curd

TABLE 1

The acidity development during the manufacture of the pasteurized-milk American Cheddar cheese and the percentage composition of the cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters

Manufacturing data	Series 1—10464			Series 2—10468		
	Lactic	L. F. ^a	Faecalis	Lactic	L. F. ^a	Faecalis
Fresh milk, titr. acid.	0.15	0.15	0.15	0.16	0.16	0.16
pH	6.64	6.64	6.64	6.54	6.54	6.54
Amount of starter (%)	2	1+1	2	2	1+1	2
Starter, titr. acid. (%)	0.74		0.64	0.77		0.61
Milk set, titr. acid. (%)	0.17	0.165	0.16	0.18	0.18	0.18
pH	6.41	6.40	6.42	6.40	6.38	6.42
Whey acid						
At cutting, titr. acid (%)	0.11	0.10	0.11	0.12	0.11	0.11
pH	6.35	6.38	6.42	6.33	6.40	6.46
Cooked, titr. acid. (%)	0.12	0.12	0.11	0.13	0.13	0.13
pH	6.22	6.28	6.35	6.21	6.33	6.32
Drawn, titr. acid. (%)	0.13	0.13	0.12	0.15	0.14	0.13
pH	6.11	6.17	6.29	6.05	6.05	6.18
Milling, titr. acid. (%)	0.52	0.45	0.38	0.50	0.38	0.25
pH	5.38	5.44	5.54	5.35	5.63	5.95
Cheese out of press						
Yield per cwt. milk (lb.)	10.4	10.4	10.4	11.0	11.5	11.9
Moisture (%)	35.9	36.9	36.3	34.9	35.8	36.9
Fat (%)	35.5	34.5	34.5	35.5	35.0	35.0
Salt (%)	1.18	1.25	1.42	1.39	1.27	1.45
Protein (%)	23.33	23.63	23.65	23.69	23.21	22.64

^a L.F.=cheese containing 1% lactic starter and 1% *S. faecalis* starter.

containing *S. faecalis* developed a more pronounced odor of good Cheddar cheese curd. This odor of good cheese curd invariably was selected by several persons.

As the cheese cured there was a gradual increase in the pH to 5.13–5.29 for cheese held at 50° F. and to 5.19–5.33 for cheese held at 60° F. (table 2). The data are not extensive enough to show any conclusive difference in acidity due to storage temperature, but a higher pH at the

warmer curing temperature seems logical. Certainly, the acidity of the ripened cheese was not affected by the starters, for the range of pH on all samples of cheese when 6 months old was from 5.13 to 5.33.

The volatile acidity of the cheese increased more rapidly at 60° F. than at 50° F. (table 2), as would be expected. The commercial lactic starter produced more volatile acidity in the cheese than the *S. faecalis*

TABLE 2

The pH, volatile acids, and water soluble proteins during curing of pasteurized-milk American Cheddar cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters

Cheese no.		pH		Volatile acids		Water-soluble proteins	
		50° F.	60° F.	50° F.	60° F.	50° F.	60° F.
				(Ml. N acid/100 g.)		(%)	(%)
				1 day old			
Lactic	10464	4.99		12.5		1.45	
Lactic	10468	4.88		12.5		2.25	
L.F.	10464	5.05		12.5		1.59	
L.F.	10468	5.01		16.7		1.74	
Faecalis	10464	5.15		12.1		1.50	
Faecalis	10468	5.12		17.5		1.54	
				2 months old			
Lactic	10464	5.07	5.13	19.5	27.5	5.77	6.68
Lactic	10468	5.06	5.14	14.7	22.9	6.01	7.28
L.F.	10464	5.10	5.15	21.4	32.4	5.55	6.40
L.F.	10468	5.17	5.22	13.6	20.7	5.65	6.00
Faecalis	10464	5.15	5.12	16.6	18.6	5.03	6.24
Faecalis	10468	5.19	5.20	16.9	17.2	5.00	6.05
				4 months old			
Lactic	10464	5.13	5.22	28.4	35.5	7.78	8.32
Lactic	10468	5.10	5.24	18.1	35.0	7.07	8.26
L.F.	10464	5.03	5.24	27.1	35.6	7.55	8.10
L.F.	10468	5.15	5.29	18.6	31.7	6.46	7.84
Faecalis	10464	5.07	5.24	23.9	29.6	7.23	7.99
Faecalis	10468		5.14	17.2	29.1	5.70	8.16
				6 months old			
Lactic	10464	5.19	5.19	27.1	43.3	7.85	8.33
Lactic	10468	5.20	5.29	27.2	39.0	8.00	9.26
L.F.	10464	5.29	5.28	29.4	39.2	7.49	8.95
L.F.	10468	5.22	5.33	22.5	36.5	7.02	8.67
Faecalis	10464	5.13	5.22	21.0	30.6	7.61	8.89
Faecalis	10468	5.19	5.31	15.8	26.2	7.10	8.61

starter, but the difference was not great. Cheese manufactured with *S. faecalis* starter and cured for 6 months at 50° F. showed practically no increase in volatile acidity. None of the samples of cheese was high in volatile acidity for, from a beginning of 12.1 to 17.5 ml. 0.1 N acid per 100 g., the volatile acidity value increased after 6 months at 50° F. up to 15.8 to 29.4 and at 60° F. up to 26.2 to 43.3.

As anticipated, the percentage of water-soluble protein increased more rapidly at 60 than at 50° F. (table 2). The two types of starters did not affect the increase in soluble proteins, which was reasonably uniform for all samples. The soluble protein in cheese cured 6 months at 50° F. ranged from 7.02 to 8.00, and that cured at 60° F. from 8.33 to 9.26.

The significant results from the use of *S. faecalis* starter are shown in the flavor scores and comments. Numerical scores were given to the nearest half point. All samples were graded as to intensity of Cheddar cheese flavor. Other comments on flavor were not made systematically, i.e., some excellent flavored samples of cheese were called excellent and others equally good were not so marked. All the comments made at scoring were entered in tables 3 and 4.

TABLE 3

Flavor development in pasteurized-milk American Cheddar cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters
(Ripened at 50° F.)

Cheese no.		Total score	Flavor ^a		Body ^b	
			Score	Remarks	Score	Remarks
1 month old						
Lactic	10464	93.0	39.0	Mild —, flat	29.0	Corky, firm
Lactic	10468	92.5	39.0	Mild —, flat	28.5	Corky, sl. crumbly
L.F.	10464	95.0	40.5	Mild +, exc., raw	29.5	Waxy
L.F.	10468	94.0	40.0	Mild +, exc., raw	29.0	Waxy, sl. crumbly
Faecalis	10464	94.5	40.0	Mild +, exc.	29.5	Waxy
Faecalis	10468	95.0	40.5	Mild, exc., raw	29.5	Waxy
2.5 months old						
Lactic	10464	94.0	40.0	Mild —, clean	29.0	Waxy, sl. firm
Lactic	10468	93.0	39.5	Mild —, sl. curd	28.5	Sl. rubbery
L.F.	10464	95.5	41.0	Mild +, clean, raw	29.5	Waxy
L.F.	10468	95.0	41.0	Mild +, clean, exc.	29.0	Waxy
Faecalis	10464	95.5	41.0	Mild, clean	29.5	Waxy, sl. rubbery
Faecalis	10468	94.5	40.0	Mild, clean, exc.	29.0	Waxy, sl. rubbery
4.5 months old						
Lactic	10464	94.5	40.0	Medium —, exc.	29.5	Waxy, sl. firm
Lactic	10468	93.0	39.0	Mild, past.	29.0	Sl. waxy, sl. firm
L.F.	10464	94.5	40.0	Medium +, exc.	29.5	Waxy
L.F.	10468	94.5	40.0	Medium, raw	29.5	Waxy
Faecalis	10464	94.0	39.5	Medium, exc.	29.5	Waxy
Faecalis	10468	94.0	39.5	Medium —	29.5	Waxy
7 months old						
Lactic	10464	93.5	39.5	Medium —, sl. flat	29.0	..
Lactic	10468	94.0	39.5	Medium	29.5	..
L.F.	10464	95.0	40.5	Medium +	29.5	Waxy
L.F.	10468	95.0	40.5	Medium +	29.5	Waxy
Faecalis	10464	94.5	40.0	Medium	29.5	Waxy
Faecalis	10468	94.5	40.0	Medium	29.5	Waxy

^a Flavor was scored with 45 as perfect. Intensity of flavor was rated mild-mild, mild +, medium —, medium, medium +, sharp —, sharp.

^b Body was scored with 30 as perfect.

Considering the cheese ripened at 50° F. (table 3), it will be noted that the cheese made with commercial lactic starter scored the lowest or possessed least flavor, whereas the cheese made with both lactic and *S. faecalis* starters scored the highest or possessed the most flavor of the

TABLE 4

Flavor development in pasteurized-milk American Cheddar cheese made with lactic, lactic plus S. faecalis, and S. faecalis starters
(Ripened at 60° F.)

Cheese no.		Total score	Flavor ^a		Body ^b	
			Score	Remarks	Score	Remarks
1 month old						
Lactic	10464	94.5	40.0	Mild	29.5	Sl. open
Lactic	10468	93.5	39.5	Mild	29.0	Firm
L.F.	10464	95.5	41.0	Medium, raw	29.5	Waxy
L.F.	10468	96.0	41.5	Medium, raw, exc.	29.5	Waxy
Faecalis	10464	94.5	40.0	Medium, raw	29.5	Waxy
Faecalis	10468	95.5	41.0	Medium, raw, exc.	29.5	Waxy
2.5 months old						
Lactic	10464	94.5	40.5	Mild +, past., flat	29.0	Sl. mealy
Lactic	10468	94.0	40.0	Mild, clean	29.0	Sl. crumbly
L.F.	10464	96.5	42.0	Medium +, clean, exc., raw	29.5	Waxy
L.F.	10468	96.5	42.0	Medium, clean, exc., raw	29.5	Waxy
Faecalis	10464	95.5	41.0	Medium, clean, raw	29.5	Waxy
Faecalis	10468	95.5	41.0	Medium —, exc., clean, raw	29.5	
4.5 months old						
Lactic	10464	94.5	40.0	Medium +	29.5	Waxy, sl. firm
Lactic	10468	91.5	38.0	Medium, burnt	28.5	Weak, sticky
L.F.	10464	95.0	40.5	Sharp —	29.5	Waxy
L.F.	10468	95.5	41.0	Medium +, exc.	29.5	Waxy
Faecalis	10464	95.0	40.5	Sharp —	29.5	Waxy
Faecalis	10468	94.5	40.0	Medium +, exc.	29.5	Waxy
7 months old						
Lactic	10464	93.0	39.0	Medium +, flat, burnt	29.0	Sl. crumbly
Lactic	10468	91.5	38.0	Medium, burnt	28.5	
L.F.	10464	94.0	39.5	Sharp —	29.5	
L.F.	10468	93.5	39.5	Medium +	29.0	
Faecalis	10464	94.0	39.5	Sharp —	29.5	
Faecalis	10468	93.5	39.5	Medium +	29.0	

^a Flavor was scored with 45 as perfect. Intensity of flavor was rated mild—, mild, mild +, medium—, medium, medium +, sharp—, sharp.

^b Body was scored with 30 as perfect.

three lots. The difference in the scores was obvious for cheese of all ages up to 7 months, when scoring was discontinued. Furthermore, the intensity of the Cheddar flavor was greatest for cheese made with both lactic and *S. faecalis* starters and was least for cheese made with lactic

starter only. The flavor of the cheese with lactic starter usually was slightly flat, whereas with the use of both starters the flavor was full, clean, and often said to be excellent and like good raw milk cheese. The flavor of cheese made with *S. faecalis* starter alone closely resembled that of cheese made with combination starter, but neither quality nor intensity of flavor always was as good. It should be noted that when the flavor of the cheese with lactic starter was especially good, the quality closely approached that of cheese made with lactic and *S. faecalis* starters. All lots of cheese cured well without developing any off-flavors for 7 months at 50° F.

The waxy, mellow body of cheese made with the combination commercial lactic and *S. faecalis* starters, and with *S. faecalis* starters alone, was evident (table 3). The improved body of these samples of cheese could be detected even after 7 months of curing. The difference was much more noticeable than might be supposed by observing the numerical scores on body.

Cheese cured at 60° F. developed the same character as that cured at 50° F., except that changes occurred more rapidly. At 50° F. no cheese developed a sharp flavor in 7 months, but at 60° F., cheese made with *S. faecalis* starter alone or in combination with lactic starter was sharp in flavor in 4.5 months (table 4). The intensity of Cheddar flavor at 2.5 months at 60° F. approximated the intensity of flavor after 4.5 months at 50° F. This agrees closely with the work of Dahlberg and Marquardt (2). At 60° F. cheese made with lactic starter began to show some deterioration in flavor at 4.5 months and was obviously deteriorated at 7.5 months. The defect was a burnt or caramelized off-flavor. The cheese made with *S. faecalis* starter alone or in combination with lactic starter was excellent after 7 months at 60° F., but the flavor quality was slightly less than at 4.5 months of age. Observations of other batches of cheese show that about 4 months at 60° F. should be the maximum forced curing before cold storage at 40° F.

DISCUSSION

The scientific literature on Cheddar cheese of the last 50 years contains many articles showing that the use of a certain bacterial culture or enzyme or of a specific process of manufacture has intensified good flavor. With minor exceptions, none of these promising results ever has been successfully used commercially over an extended territory. Therefore, it is with some reluctance that the authors publish these data, but the results are of scientific value irrespective of commercial usage. This study is the first to embody successfully the use of large inoculations of a special culture into pasteurized milk to produce good, high flavored

American Cheddar cheese in a short curing period. Although the flavor is like that of raw milk cheese, it never develops the very "bitey" or "snappy" flavor that stings on the upper palate of the mouth, and is so typical of old raw milk cheese. Rather, the flavor is full and pronounced without being astringent or without having any rancid or other foreign flavor. Obviously, the milk must be of good flavor before pasteurization.

Good Cheddar cheese was made in these experiments with pasteurized milk containing only a commercial lactic starter. A better Cheddar cheese was made using *S. faecalis* starter alone, so this is positive proof that ordinary lactic starter is not necessary for making good Cheddar cheese. The best Cheddar cheese was made using both starters together, indicating symbiotic action among the bacteria in the two starters in producing a maximum of flavor.

In these experiments no time was given for the starter to develop before adding rennet, and the authors actually favor an hour at 86° F. for the starter to work in the milk before setting. Under such conditions about 0.5 to 0.75 per cent of lactic starter and 1 per cent of *S. faecalis* starter gave desired acid production.

S. faecalis has characteristics of special significance in cheese. It grows at 106° F. in the cheese vat and at 50° F. in the curing room. It ferments lactose rapidly enough to be used as a starter, providing a proper strain is selected and developed. It grows anaerobically in cheese, utilizing lactates as sources of energy. It is nonproteolytic and does not produce gas or objectionable flavors. It grows at the pH and the salt concentration present in cheese.

SUMMARY

A strain of *Streptococcus faecalis* which rapidly fermented lactose was isolated. It was used as a starter for American Cheddar cheese made from pasteurized milk of excellent quality.

The *S. faecalis* starter produced acid in milk somewhat slower than a commercial lactic starter but rapidly enough for cheese making. The cheese made with *S. faecalis* developed a normal acidity, slightly lower total volatile acidity, and the same water-soluble protein level as did cheese made with a commercial lactic starter. More Cheddar flavor of better quality developed in the cheese made with *S. faecalis*, and the body of the cheese was more mellow and waxy than the cheese made with lactic starter.

American Cheddar cheese with the best flavor of highest intensity was made by using both commercial starter and *S. faecalis* starter in the same pasteurized milk. The flavor was pronounced, clean, good Cheddar but not snappy.

S. faecalis starter hastened the ripening of Cheddar cheese. A well-ripened cheese of medium flavor intensity was produced in 4.5 months at 50° F. and in 2.5 months at 60° F. when *S. faecalis* starter was used with the usual lactic starter. With commercial lactic starter, the same approximate intensity of flavor, of lower quality, was developed in 7 months at 50° F. and in 4.5 months at 60° F. The results indicate that after these curing periods, the cheese should be held at cold curing temperatures.

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THE GROWTH AND SURVIVAL OF *STREPTOCOCCUS FAECALIS* IN PASTEURIZED MILK AMERICAN CHEDDAR CHEESE¹

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In a previous paper (1) it was shown that the addition of *Streptococcus faecalis* starter to pasteurized cheese milk increased the rate of ripening and improved the flavor of American Cheddar cheese. Such an effect naturally directed the attention of the authors to the growth characteristics of this organism in cheese.

It is well known that the bacteria normally found in commercial lactic cheese starters do not survive for any great length of time in cheese. *S. faecalis*, on the other hand, is considered to be a rugged type of organism, able to survive and grow under conditions which would soon destroy many other types. As American Cheddar cheese in many respects affords conditions unfavorable for growth of most bacteria, it would be interesting to observe the degree of adaptation that *S. faecalis* could make in such an environment. That this organism commonly is found in cheese has been noted by several investigators. Sherman and Stark (5) found *S. faecalis* in 1-day-old Swiss cheese, while Foster *et al.* (2) found large numbers of these bacteria in ripening Brick cheese. Tittsler *et al.* (6) stated that enterococci were one of the predominant bacterial types in ripening Cheddar cheese made from pasteurized milk.

Up to the present, very little study has been made of the course of growth and of the survival period of *S. faecalis* in Cheddar cheese. Data of this nature should aid in an understanding of the effect of this organism upon cheese flavor development as well as provide general information which will be required as knowledge of the relationship of *S. faecalis* to foods becomes more apparent. A study covering the foregoing phases was conducted.

EXPERIMENTAL METHODS

Methods used consisted of the total plate count using standard tryptone-glucose-extract-skim milk agar, a selective medium plate count for enterococci, and conventional physiological identification tests for enterococci. The selective medium was that developed by White and Sherman (7) for the determination of enterococci in milk. It contains 0.5 per

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cent glucose, 0.5 per cent tryptone, 0.5 per cent yeast extract, 1.5 per cent agar, 0.03 per cent sodium azide, and 325 Oxford units of penicillin per l. Tests employed for the identification of the enterococcus group were those suggested by Sherman (4) as being very important for differential purposes. They included growth at 10° C. and 45° C., rapid reduction in litmus milk, and growth in broth containing 6.5 per cent sodium chloride. In addition, microscopic observations were made. All plates were incubated at 32° C. for 4 days. Samples for plating were prepared by aseptically grinding 3 g. of cheese with 27 ml. of warm 2 per cent sodium citrate solution until the cheese was well emulsified. Dilutions then were made from this cheese solution.

RESULTS

Total and selective medium counts of milk, starters, and fresh curd. Results shown in this work were obtained on a series of three cheese. All cheese were made from milk pasteurized at 143–145° F. for 30 minutes, with the raw milk being obtained from the Cornell University herd. Twelve hundred pounds of milk were divided into three equal portions and made into cheese. The cheese obtained was the series *L* 10468, *LF* 10468 and *F* 10468 referred to in a previous paper (1) in which *L* was made with 2 per cent commercial lactic starter, *LF* with 1 per cent commercial lactic starter and 1 per cent *S. faecalis* starter, and *F* with 2 per cent *S. faecalis* starter. The cheese were ripened at 50 and at 60° F.

Bacterial counts, using standard agar and penicillin-sodium azide medium, were made of the original raw and pasteurized milk, and starters. Although the selective medium was used to separate the enterococci, no attempt was made at this stage to identify the enterococcus colonies by additional tests. Results of these counts are shown in table 1. The two different types of starters showed relatively high total bacterial counts. The regular commercial starter showed a total bacterial count of 350 million per ml., while the *S. faecalis* starter had a total bacterial count of 950 million per ml. Results with the selective medium showed that no enterococci were present in the regular commercial starter, while approximately 94 per cent of the total count of the *S. faecalis* starter grew in the selective medium.

The original raw milk was of high quality, having a total bacterial count of 5,700, while the pasteurized milk had a low bacterial count of 500 per ml. The numbers of bacteria growing on the selective medium were very small in either milk. The total bacterial counts after the addition of the starters ranged from 5 million to 16 million, whereas the selective medium counts ranged from 70 to 12 million.

The next counts were taken on the cheese curds just before salting.

Results are listed in table 2 under 0 days. Beginning with this section and continuing through, with a few exceptions, 20 colonies from the plates of each cheese were isolated and cultured from plates containing the selective medium. These bacteria then were identified as to whether or not they were enterococci, using the tests enumerated previously.

Total and selective medium counts of cheese ripened at 50° F. Of the cheese ripened at 50° F. (table 2), cheese *L*, containing 2 per cent lactic starter, had its highest total bacterial count, 300 million per g., within the first 2 days, after which the bacterial population decreased rapidly to the

TABLE 1

Bacterial counts on starters and cheese milk containing commercial lactic starter organisms and S. faecalis organisms

Milk	Total bacterial counts per ml. on standard agar	Bacterial counts per ml. on penicillin-azide agar
Commercial starter	350,000,000	1
<i>S. faecalis</i> starter	950,000,000	890,000,000
Raw milk	5,700	320
Past. milk (143-145° F. for 30 min.)	500	50
<i>L</i> 10468—past. milk set at 86° F. containing 2% com. starter	5,000,000	70
<i>LF</i> 10468—past. milk set at 86° F. containing 1% com. starter and 1% <i>S. faecalis</i> starter	11,500,000	6,400,000
<i>F</i> 10468—past. milk set at 86° F. containing 2% <i>S. faecalis</i> starter	16,000,000	12,000,000

low total count of 1,200,000 per g. at the end of 23 days and then gradually increased to a total count of 26 million at the end of 180 days. Cheese *LF*, containing 1 per cent lactic starter and 1 per cent *S. faecalis* starter, had its highest total count, 1,150 million per g., at the time of salting the curd. The number of bacteria then decreased very slowly over the ripening period of 180 days to a low of 305 million. Cheese *F*, containing 2 per cent *S. faecalis* starter, on the other hand, had a high total count of 1,790 million at the time of salting, but this high count was maintained at the same level for 60 days, after which it slowly decreased to 855 million at the end of 180 days.

When the selective penicillin-azide medium for enterococci was used on the cheese ripened at 50° F., the following results, outlined in table 2, were obtained. The selective medium bacterial count for cheese *L* was lowest during the first 2 days. Just prior to salting of the cheese, the bacterial

count was 300, with 18 of the 20 colonies being identified as enterococci. From this low point, the bacteria in this control cheese increased to a peak of 23 million per g. However, it can be seen clearly from the summary of identification tests (table 2) that the increase in numbers was not a

TABLE 2

Bacterial counts of pasteurized-milk American Cheddar cheese (Series 10468) made from commercial starter, S. faecalis starter, and a mixture of the two, and ripened at 50° F.

Cheese a	Age . (days)	Total bacterial count per g. on standard agar	Bacterial count per g. on penicillin-azide agar	Positive identification of enterococci (from 20 picked colonies)
L	0	300,000,000	300	18
LF	0	1,150,000,000	520,000,000	19
F	0	1,790,000,000	1,370,000,000	20
L	2	320,000,000	100	
LF	2	1,070,000,000	490,000,000	
F	2	1,890,000,000	1,100,000,000	
L	11	60,000,000	5,000	4
LF	11	920,000,000	500,000,000	19
F	11	1,750,000,000	1,310,000,000	20
L	23	1,200,000	14,000	
LF	23	686,000,000	389,000,000	
F	23	1,560,000,000	1,076,000,000	
L	34	2,900,000	295,000	2
LF	34	740,000,000	390,000,000	19
F	34	1,810,000,000	1,065,000,000	20
L	60	11,000,000	2,700,000	6
LF	60	480,000,000	345,000,000	20
F	60	1,630,000,000	1,085,000,000	19
L	120	44,000,000	23,000,000	0
LF	120	366,000,000	290,000,000	19
F	120	970,000,000	740,000,000	18
L	180	26,000,000	11,000,000	0
LF	180	305,000,000	250,000,000	19
F	180	855,000,000	675,000,000	20

a L = 2% regular lactic starter in cheese milk.

LF = 1% regular lactic starter in 1% *S. faecalis* starter in cheese milk.

F = 2% *S. faecalis* starter in cheese milk.

result of an increase in enterococci but rather of another type or types of bacteria able to multiply on the selective medium. Further examination of these organisms showed them to be of the genus *Lactobacillus*.

In cheese LF, the highest number of enterococci, 520 million per g., was found in the curd prior to salting. The numbers of these bacteria

were maintained at almost 70 per cent of this level for 60 days; while at the end of 180 days there was a decline of about 50 per cent. Almost all the bacteria isolated were of the enterococcus group.

Cheese *F* at 50° F. had the highest enterococcus count, 1,370 million per g., just before the curd was salted, and this count was maintained at

TABLE 3

Bacterial counts of pasteurized-milk American Cheddar cheese (Series 10468) made from commercial starter, S. faecalis starter and a mixture of the two, and ripened at 60° F.

Cheese ^a	Age	Total bacterial count per g. on standard agar	Bacterial count per g. on penicillin-azide agar	Positive identification of enterococci
	(days)			(from 20 picked colonies)
L	0	300,000,000	300	18
LF	0	1,150,000,000	520,000,000	19
F	0	1,790,000,000	1,370,000,000	20
L	2			
LF	2			
F	2			
L	11	50,000,000	36,000	3
LF	11	960,000,000	450,000,000	19
F	11	1,680,000,000	1,330,000,000	20
L	23	3,200,000	2,700,000	
LF	23	680,000,000	420,000,000	
F	23	1,490,000,000	1,030,000,000	
L	34	14,000,000	5,500,000	1
LF	34	630,000,000	385,000,000	18
F	34	1,600,000,000	1,020,000,000	20
L	60	50,000,000	26,000,000	0
LF	60	450,000,000	305,000,000	18
F	60	875,000,000	750,000,000	20
L	120	61,000,000	28,000,000	0
LF	120	150,000,000	115,000,000	18
F	120	530,000,000	450,000,000	19
L	180	35,000,000	13,500,000	0
LF	180	61,000,000	36,000,000	13
F	180	165,000,000	90,000,000	20

^a L = 2% regular lactic starter in cheese milk.

LF = 1% regular lactic starter in 1% *S. faecalis* starter in cheese milk.

F = 2% *S. faecalis* starter in cheese milk.

the same level for 60 days, after which it decreased to 675 million at the end of 180 days. Practically all the colonies isolated by means of the selective medium belonged to the enterococcus group, and, as only *S. faecalis* was added, presumably all or almost all of this species made up the bacterial count of cheese *F*.

Total and selective medium counts of cheeses ripened at 60° F. The bacterial counts of cheese ripened at 60° F. compared to those of the same lots of cheese ripened at 50° F. showed many similar trends (table 3). In control cheese *L*, the total bacterial count showed a rapid decrease from a high of 300 million to a low of 3 million per g. at the end of 23 days. This was followed by a steady increase until at the end of 120 days the total bacterial count was up to 61 million. Two months later the numbers of bacteria had decreased to 35 million. In cheese *LF* a high total bacterial count of 1,150 million was obtained on the curds just prior to salting. The total count then gradually decreased to 450 million at the end of 60 days, while at the end of 180 days the bacterial count had gone down to 61 million. Cheese *F* showed results in line with those exhibited by cheese *LF*, going from a high of 1,790 million in the curd to a low of 165 million at 180 days.

Selective medium counts made on these cheese ripened at 60° F. (table 3), showed control cheese *L* with a low initial count of 300, followed by a steady increase to 28 million at the end of 120 days and a drop to 13,500,000 at 180 days. This increase in cheese *L* was not due as much to enterococci as to lactobacilli. On the other hand, cheese *LF* had its highest enterococcus count just before salting, 520 million per g., and this population decreased to 36 million at 180 days. At the end of 60 days of ripening, 305 million enterococci per g. still were present. In this connection, cheese *F*, with *S. faecalis* starter only, showed counts which were maintained for long periods of time. Starting with an initial selective medium count of 1,370 million, this cheese still had a count of 750 million at the end of 60 days, and then dropped to 90 million at 180 days.

A comparison of tables 2 and 3 shows that at 50 and at 60° F. the trends of bacterial growth and survival were very similar; the difference that existed showed up as a more rapid decline in bacterial population at 60° F., a result which was expected.

A duplicate experiment made on cheese manufactured a month later, produced data of strikingly similar nature. These data are not included in this paper because they would only provide repetition of the initial observations.

DISCUSSION

A study was made of the growth and survival of *Streptococcus faecalis* in pasteurized milk American Cheddar cheese over a 6-month ripening period at 50 and at 60° F. Control cured cheese made from pasteurized milks and containing only regular lactic cheese starter had the lactobacilli as their predominating organisms. These results do not agree with those of Tittsler *et al.* (6), who stated that enterococci were the predom-

inating organisms in pasteurized milk cheese. However, it is pointed out that the milk used in the present study was of very high quality, and cheese made commercially from pasteurized milk was not included. Development of lactobacilli in large numbers late in the ripening period of Cheddar cheese was noted very early by Hastings *et al.* (3) and by other investigators.

The observation that the selective medium developed by White and Sherman (7) for the separation of enterococci actually allowed bacteria of the lactobacillus group to grow confirms the earlier findings of White and Sherman (8). These investigators found that in cheese certain species of lactobacilli were able to grow in the selective medium. Where enterococci are predominant, this method of selection is very useful and surprisingly consistent in its ability to recover the organism. In the work involving cheese *F*, where there was a vast number of *S. faecalis* organisms, the selective medium was able to recover on the average about 73 per cent of the total bacterial count as enterococci. This is a good recovery when one considers the many opportunities that exist for the elimination of the less resistant bacteria in work of this nature.

Streptococcus faecalis proved able to adapt itself well to the environment provided by Cheddar cheese when added as a starter. Its best growth occurred in the milk and curd up to salting, and it was able to grow and survive in large numbers after 180 days of ripening at 50 and at 60° F. This characteristic resistance to destruction in cheese further strengthens the authors' belief that this organism is instrumental in developing increased flavor in cheese.

SUMMARY

Three lots of milk pasteurized at 143–145° F. for 30 minutes were made into American Cheddar cheese. These lots contained, respectively, 2 per cent commercial lactic starter, 1 per cent commercial lactic starter plus 1 per cent *Streptococcus faecalis* starter, and 2 per cent *S. faecalis* starter. A selective penicillin-azide medium was used to count and isolate the enterococci.

In 1-day-old cheese made with commercial lactic starter, the number of bacteria growing on the selective medium was small, 300 per ml., but these gradually increased to 23 million per ml. at the end of 120 days at 50° F., and to 28 million per ml. after 120 days at 60° F. At the end of 180 days the counts on the selective medium had decreased to 11 million and 13 million per ml. at 50 and 60° F., respectively. In this cheese most of the increase was due to lactobacilli and not to enterococci.

When *S. faecalis* was used as a starter for pasteurized milk American Cheddar cheese, the highest enterococcus count was found to exist in the

cheese curds just prior to salting, the count being 500 million per g. for cheese containing 1 per cent commercial lactic starter plus 1 per cent *S. faecalis* starter, and 1,370 million per g. for the cheese containing 2 per cent *S. faecalis* starter.

S. faecalis was able to grow and survive in Cheddar cheese in large numbers for a considerable period of time, both at 50 and 60° F. At 50° F., cheese made with both lactic and *S. faecalis* starters still gave counts of 345 million per g., and cheese made with *S. faecalis* starter gave counts of 1,085 million per g. at the end of 60 days, whereas at 60° F. the former cheese contained 305 million per g. and the latter cheese contained 750 million per g. at the end of 60 days. At the end of 180 days the numbers of bacteria in the cheese growing on the selective medium had decreased, although considerable numbers still were present.

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THE TYRAMINE CONTENT OF CHEESE¹

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The process of cheese ripening produces a variety of nitrogenous decomposition products, some of which must exert an important influence upon the ripening process. Yet relatively little is known concerning the nature of many of these compounds, their concentration in cheese, their specific rôle in ripening, and their nutritional value. For example, information is relatively meager on the amino acids freed in cheese during ripening, and even less is known concerning the respective amines which may be formed from these amino acids.

This paper deals with a small portion of this complex problem in that a quantitative study on cheese was made of the amine derived from tyrosine. This breakdown product from tyrosine is *p*-hydroxyphenylethylamine and very commonly is referred to as tyramine. No information has been available to indicate its quantitative concentration in cheese, with the single exception of an Emmenthal cheese.

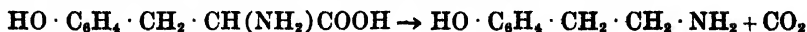
Tyramine is an alkaloid of the aromatic amine type. It can be produced by heating tyrosine with a high boiling solvent such as diphenylamine or by bacterial decomposition of tyrosine. Often it occurs in decaying protein and it also is found in ergot and mistletoe. Tyramine has a boiling point of 179–181° C. (8 mm.) and a melting point of 161° C. When injected subcutaneously or intravenously, it has the property of contracting the peripheral blood vessels, thus causing an increase in blood pressure, and for this reason it is used rather frequently in medicine. Gale (6) found the optimum production of tyramine by bacterial cells to occur at pH 4.5 to 5.5, which is in the pH range of normal American Cheddar cheese.

Tyramine was first discovered in cheese in 1903 by Van Slyke and Hart (8) in their research to show the source of carbon dioxide in cheese. They made two small batches of Cheddar cheese from fresh milk and from fresh milk to which chloroform had been added. Of specific interest in their study was the accumulation of relatively large amounts of tyrosine and no tyramine in the chloroformed cheese (a low acid cheese) after curing for 32 weeks at 15.5° C. (60° F.) as compared with lesser amounts of tyrosine and positive tests for tyramine in the normal cheese. This conversion of tyrosine to tyramine was thought to be due to bacteria. They cited the research of Emerson (5), published the previous year, which established

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that the aqueous extract of the pancreas converted tyrosine into tyramine with liberation of carbon dioxide by the following reaction:



An abnormal Emmenthal cheese was found in 1909 by Winterstein and K  ng (10) to contain tyramine. The authors did not state the reason for considering this cheese to be abnormal, but they found the tyrosine content to be abnormally low for a well-ripened cheese. They believed that bacteria probably converted the tyrosine into tyramine. Later Winterstein (9) found traces of tyramine in a skim milk Emmenthal cheese.

In 1914 Ehrlich and Lange (4) reported the presence of tyramine in samples of Roquefort, Camembert and Emmenthal cheese. The tyramine content of the Emmenthal cheese was found to be 1.08 g. in 1.8 kg. of cheese, or 0.06 per cent. When the cheese was inoculated into a bacterial culture medium containing tyrosine, tyramine was produced. A culture was isolated which produced tyramine by this method, but it did not produce tyrosol or *p*-hydroxyphenyllactic acid. This culture which produced acid in milk belonged to the colon group of bacteria. The literature includes the analysis for tyramine of only one Cheddar, one Roquefort, one Camembert and three Emmenthal cheeses. All samples tested showed the presence of tyramine, but of these six samples, only one, an Emmenthal cheese, was analyzed quantitatively.

Methods for the separation and estimation of tyramine have been known for some time, but only in recent years have newer methods been introduced which can be applied on a large scale to the estimation of this compound in such products as cheese. Henze (7) developed a quantitative method for obtaining and separating tyrosine and tyramine from cephalopods by ether extraction. With this method he isolated tyrosine and tyramine from the salivary gland of *Octopus macropus* and determined the compounds colorimetrically by the Millon reaction. Recently Bellamy and Gunsalus (1, 2), by using a continuous ether extractor and a colorimeter, adapted and applied this method to the determination of tyrosine and tyramine in bacterial cultures in their study on tyrosine decarboxylase systems. Utilizing the knowledge obtained by the foregoing investigators, an applied method for determining tyramine in cheese was evolved. With this method it was possible to test quantitatively a large number of cheeses.

EXPERIMENTAL METHODS

The principle of this method is that the phenolic hydroxyl group, which is characteristic for tyrosine and tyramine, will react to form a positive Millon test under the proper conditions. Separation of tyramine from tyrosine is based on the fact that tyramine can be extracted by ether under mildly alkaline conditions, while tyrosine is insoluble in ether. Both are

insoluble in acid ether, while phenols are soluble. The method as applied to cheese is described.

Preparation of sample. Fifteen grams of cheese were ground in a mortar with a small amount of warm ($45^{\circ}\text{C}.$) 2 per cent sodium citrate solution. After the cheese was well emulsified, the solution was transferred quantitatively to a 250-ml. volumetric flask and enough sodium citrate solution was added to bring it to the 250-ml. mark after cooling to $25^{\circ}\text{C}.$ The contents of the volumetric flask were transferred to a standard 300-ml.

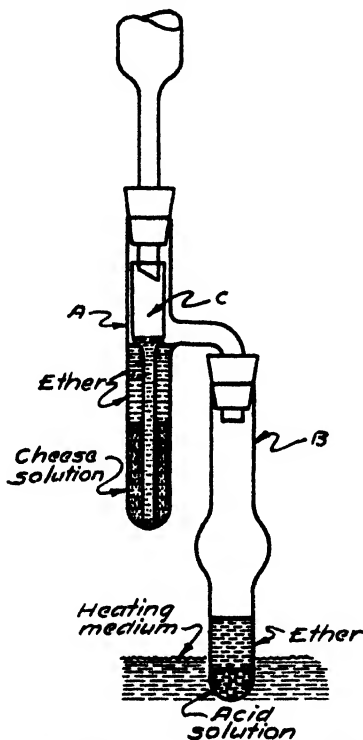


FIG. 1. A continuous extractor for separating tyramine from tyrosine. A, extraction tube (200×25 mm.); B, receiver tube (200×25 mm.); C, glass thimble. Condenser capacity—300 mm.

pyrex flask and heated to $80^{\circ}\text{C}.$ for 15 minutes in a water bath to destroy the decarboxylases and then cooled to $25^{\circ}\text{C}.$ After cooling, an 18-g. sample obtained by using a calibrated pipette was transferred to the extraction tube (A) of the continuous ether extractor shown in figure 1. This extractor, very similar to the one proposed by Wooley (11), was used by Bellamy and Gunsalus (1, 2) in their work on bacterial decarboxylases.

Acid extraction of cheese. To the receiver tube (B, fig. 1) of the ex-

tractor, 5 ml. of M/50 sulfuric acid were added. This acid solution is used to trap the tyramine when it comes over with the ether. The glass thimble (C, fig. 1) was placed in the extraction tube and then ethyl ether carefully was added to both the extractor and receiver tube. This was done until a 3-cm. layer accumulated in the receiver and until the ether just reached the side arm in the extractor tube. The two sections were attached to each other by means of cork connections and then attached to a condenser. Gentle heating of the ether in the receiver tube was accomplished by the use of an oil bath. Extraction under acid conditions was carried on for 5 hours to remove a large portion of the fat and fatty acids and any phenols that might be present. This extraction, due to the influence of the sodium citrate, was carried at slightly above the pH of most normal Cheddar cheese, or about pH 5.5-5.8.

Alkali extraction of cheese. The receiver tube was emptied, washed, and again filled to its former level with 5 ml. of M/50 sulfuric acid and sufficient ethyl ether and attached to the extraction section. The entire extraction unit then was taken from the oil bath, and enough of a solution of 10 per cent sodium carbonate was added to the thimble in the extractor tube to make the solution to be extracted slightly alkaline to phenolphthalein. The quantity required varied from 0.4 to 1.0 ml. No phenolphthalein actually was added to the extractor tube as it would be extracted by ether and would give a positive test. To find the proper amount of alkali required without adding phenolphthalein to the unit, an Erlenmeyer flask containing 18 g. of the cheese solution and phenolphthalein was used and the solution in it neutralized. The same amount of alkali required for this preliminary neutralization was added to the thimble. Extraction was resumed and continued until all the tyramine was obtained. This usually took about 42 hours, depending on the nature of the cheese. To assure complete extraction, analyses were made on the contents of the receiver tube after 30 hours and then at 12-hour intervals until the pink color no longer was produced.

After each of these extraction periods the receiver tube was disengaged from the extractor tube and the entire ether-acid mixture was cooled slightly and poured slowly into a graduated test tube. The test tube was placed into an oil bath and the ether carefully boiled off. The acid solution remaining was cooled to 25° C., and the volume noted and tyramine analyses made. The receiver tube again was filled with acid solution and ether, and the extraction resumed.

Measurement of color. One milliliter of the acid solution containing the tyramine was pipetted into a colorimeter tube (calibrated 18 × 150 mm. pyrex test tube). Three milliliters of 95 per cent acetic acid were added, followed by 2 ml. of a mercuric sulfate reagent (10 per cent mercuric sulfate in 5 per cent sulfuric acid) and the tube well agitated. This mixture

was heated for 3 minutes in boiling water, cooled and mixed. The tube then was placed in a Coleman no. 11 spectrophotometer and read at 500γ against a reagent blank set at $G = 100$. After this turbidity reading (L_1) if any, was recorded, 1.0 ml. of fresh 0.5 per cent sodium nitrate (prepared fresh daily) was added and well mixed, and the tube read at 500γ after 15 minutes at room temperature against a reagent blank to which sodium nitrate solution had been added. The second reading with the color-producing compound was labeled L_2 .

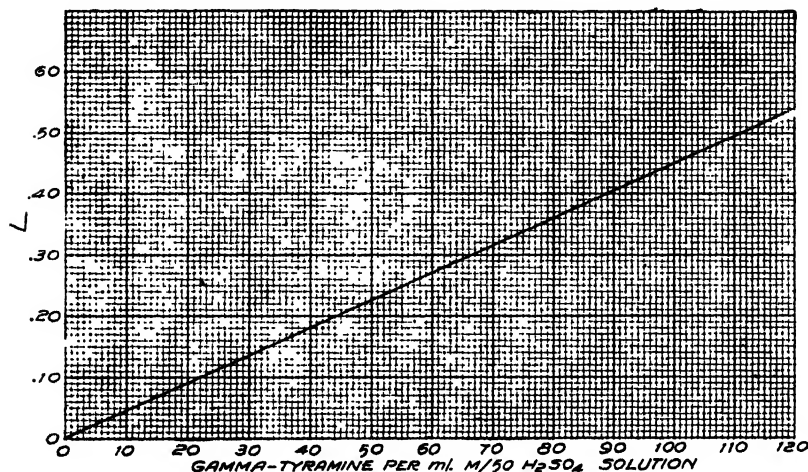


FIG. 2. Standard curve for tyramine using the Coleman spectrophotometer.

Calculations. Calculations required for this method are as follows:

$$L = 2 - \log G$$

$$L_2 - L_1 = L \text{ (proportional to tyramine concentration)}$$

G = galvanometer reading

L_1 = value of turbidity

L_2 = value for color

To find the amount of tyramine in the sample, one of two things can be done. Reference can be made to the standard curve (fig. 2), or the tyramine content can be calculated by using a constant K , which has been obtained at different levels. To use K , the standard curve must go through the origin.

$$K = \frac{\text{gamma in standard sample}}{L \text{ for same sample}}$$

$$\text{Gamma in standard sample} = KL$$

The final standard is brought to gammas of tyramine per gram of cheese, after considering all dilution factors. In this work all values are based on tyramine and not tyramine hydrochloride.

RESULTS

The recovery of added tyramine from cheese. In order to test the ability of this applied method to recover tyramine from cheese effectively and at the same time to prevent tyrosine from being extracted, a number of recovery experiments were performed. The results from several experimental trials indicated that when pure tyrosine was added to water or to a cheese solution, no amount of extraction, either at an acid or alkaline pH, would bring over the tyrosine. On the other hand, when various amounts of tyramine in the form of tyramine hydrochloride were added to a cheese solution, which then was made mildly alkaline with 10 per cent sodium carbonate, it was found (table 1) that practically all the added tyramine was recovered under the conditions of extraction and color

TABLE 1

The recovery of added tyramine from cheese (5468 Fe)
(5-hr. acid ether extraction + 40-hr. alkali ether extraction)

Tyramine added	Tyramine found in cheese	Tyramine recovered
($\gamma/g.$)	($\gamma/g.$)	(%)
0	1427
790	2191	97.0
1580	3008	100.0

estimation previously described. In this recovery experiment 5 hours of acid ether extraction and 40 hours of alkali ether extraction were required for complete recovery. For some cheese solutions where smaller amounts of tyramine were added, complete recovery was attained in a shorter length of time.

The tyramine content of 25 Cheddar cheeses. Analyses for tyramine were conducted on 25 commercial American Cheddar cheeses. These samples were obtained from New York, Wisconsin, Illinois, and Missouri and consisted of both raw and pasteurized milk cheese. The ages of these cheese ranged from 2 months to 3 years. No attempt was made to show here the effect of such factors as treatment of milk or cheese and the effect of age upon the tyramine content, as these topics will form the basis of subsequent papers. The high, average, and low tyramine concentrations of 25 American Cheddar cheeses are shown in table 2.

The tyramine content of Cheddar cheese can be much greater than that listed in the table. One very old Cheddar cheese, not included in the group of 25 cheeses because it was not manufactured in a commercial plant, was found to contain 2,330 γ of tyramine per g., or 0.223 per cent. Data shown

here make it quite apparent that practically all commercial American Cheddar cheese must contain tyramine in a wide range of concentration.

The tyramine content of miscellaneous types of cheese. A number of cheeses representing diversified types were subjected to analyses for tyramine. The results of these analyses are shown in table 3. All samples within a type variety were purchased from different sources and at different times of the year. It was assumed from the beginning that the values shown here do not necessarily typify any particular variety of cheese, as factors tending to shift these values always are present. Data shown in table 3, however, do give some idea as to the tyramine content of an assorted group of cheeses. Here again, as with the Cheddar cheeses, a wide range exists not only between varieties but also between samples of similar types. The smallest amount of tyramine, 48 γ per g., occurred in a Roquefort, while the largest amount, 1,683 γ per g., occurred in a Liederkrantz cheese.

TABLE 2

The concentration of tyramine in twenty-five commercial Cheddar cheeses

Cheese	Tyramine	
	(γ /g.)	(%)
Highest	1199	0.1199
Lowest	25	0.0025
Av.	384	0.0384

Two different samples of Liederkrantz both gave high concentrations of tyramine. This is undoubtedly a result of the character of this cheese, where early and extensive decomposition of proteins occurs. The Limburger sample, which is considered similar to Liederkrantz in its decomposition properties, had a relatively low value. Further examination of this sample showed it to be very atypical in that it was not broken down in body and that it resembled a Brick cheese more than a Limburger.

The isolation, purification and identification of the dibenzoyl derivative of tyramine from Cheddar cheese. A series of tyramine extractions was conducted on several Cheddar cheeses which were considered to have large concentrations of tyramine. Sufficient extractions were made from fresh samples of cheese to provide a volume of 500 ml. of N/50 sulfuric acid calculated to contain about 35 mg. tyramine. The acid solution (about pH 1) then was evaporated *in vacuo* to approximately 40 ml., washed with ethyl ether to remove any fat, and then centrifuged to remove other impurities.

The method for obtaining a dibenzoyl derivative of tyramine as described by Gale (6) was followed. Solid sodium bicarbonate was added until the pH of the solution was brought to 7.5-8.0 and the mixture was

cooled in ice to 10–12° C. Benzoyl chloride then was added, with vigorous shaking, a few drops at a time until about 3 mol. equivalents had been added, the pH being maintained in the region of 8 by the addition of solid sodium bicarbonate. This mixture was left overnight in a cold room. The following day a precipitate had formed and was removed by centrifuging. It was purified by extracting with hot absolute alcohol. The dibenzoyl

TABLE 3
*The concentration of tyramine in an assorted
group of cheeses*

Cheese	Tyramine	Tyramine
	($\gamma/g.$)	(%)
Edam ^a	214	0.0214
Edam	100	0.0100
Roquefort ^a	48	0.0048
Blue ..	49	0.0049
Blue ..	266	0.0266
Limburger	204	0.0204
Liederkrantz	1226	0.1226
Liederkrantz ..	1683	0.1683
d'Oka ^a	310	0.0310
d'Oka ^a	158	0.0158
Gouda ^a	95	0.0095
Gouda	54	0.0054
Brick	194	0.0194
Munster	110	0.0110
Swiss	50	0.0050
Swiss	434	0.0434
Romano ^a	197	0.0197
Argenti ^a	188	0.0188
Camembert	125	0.0125
Mild process ^b	164	0.0164
Cheese food	125	0.0125

^a Imported cheeses.

^b Cheddar.

tyramine was recrystallized once from dilute alcohol and its melting point determined on a hot-stage microscope. A sample of dibenzoyl tyramine was prepared in the same manner from highly purified Eastman Kodak tyramine hydrochloride and the melting point of the derivative taken by the hot-stage microscope. Gale (6) reported that the dibenzoyl tyramine obtained in his studies had a melting point of 171–172° C. (corr.). The derivative from the present cheese and from the known pure tyramine melted at 170–172° C. (corr.) and a melting point of the mixed samples was 169–172° C. (corr.). These data confirm that tyramine was the chemical substance being extracted from the cheese.

DISCUSSION

It has been possible to show the presence and concentration of tyramine (*p*-hydroxyphenylethylamine) in a large number of ripened types of cheese. As tyramine can be derived from tyrosine by bacterial decarboxylases, a prerequisite of tyramine production in cheese is the presence of free tyrosine. The observations of Dorn and Dahlberg (3) that the white particles in ripened Cheddar cheese actually are made up largely of tyrosine fully satisfies this condition.

The fact that tyramine was found in practically all commercial cheeses examined is not as surprising as the concentrations found. Past concepts that tyramine occurs in normal cheese only in traces, if at all, will have to be revised. A concentration of from 0.08 to 0.12 per cent cannot be considered a trace. In these analyses it was found that 5 out of 25 commercial American Cheddar cheeses fell in the above group. Because of the very unique properties of tyramine, the implications and significance of the presence of such amounts in cheese should pose some very interesting questions for future study and should prove to be a fertile field for investigation.

Compounds other than tyramine and tyrosine, but containing the same characteristic phenolic hydroxyl grouping, if present, also would give a positive Millon test. These compounds would include tyrosol, *p*-hydroxyphenyllactic acid, *p*-hydroxyphenylacetic acid, thyroxine, dopa, phenolphthalein, phenol, salicylic acid and thymol. However, practically all of these compounds can be ruled out either as being insoluble in ether or as never having been reported to be found in cheese. If free phenol were present, it would be removed by the acid ether extraction. Nevertheless, to make certain that tyramine actually was being obtained, a dibenzoyl derivative of it was isolated and purified from cheeses after standard extraction by the method.

The initial 5-hour acid extraction was introduced in the method to remove fat and fatty acids. At the end of this period most of the fat will be extracted, thus ruling out fat as an experimental factor, and no tyramine will be extracted. This was true for all Cheddar cheeses which are extracted at a pH range of 5.0 to 5.8, but in the case of well broken-down cheeses such as Liederkranz, where the pH was higher, some tyramine was recovered. In the actual analyses these initial recoveries of tyramine were added into the total.

The method as applied to cheese usually produced results on duplicate samples with an experimental error of less than 3 per cent. Results below 50 γ per g. produced a larger experimental error. However, as the range of values was from 25 γ to more than 2,000 γ , this variation was not important.

Several precautions should be stressed. Unless the extraction rate is

very slow at the beginning, a stable sludge or emulsion will occur in the ether of the extraction tube, finally increasing in amount to such a point that it will carry over into the receiver tube. If this happens, a complete re-extraction must be begun. To overcome this emulsification effect it has been found feasible to conduct the extraction for the first 12 hours at a rate of not more than 40 drops per minute. If an emulsion has formed but has not gone through the side arm, the extraction tube may be disengaged and the thimble removed, after which the extraction tube is placed in the warm oil until the emulsion has settled. After slight cooling of the tube, the thimble is replaced, more ether is added, and the extraction process is continued. Other factors which have been observed to aid in forming this emulsion are the presence of green cheese and the extraction of greater amounts of cheese than that recommended here.

The final acid solution in the receiver tube usually is clear. If the solution contains protein particles carried over by the emulsion, usually easily visible, the results will be in error and cannot be used. Also, the ether must be completely boiled off; failure to do this will increase turbidity and will provide erroneous results. A blank should be run on reagents.

SUMMARY

A method for separating tyramine from tyrosine and for estimating the concentration of the former substance was applied to cheese. This method involved the use of a continuous ether extractor and the employment of the Millon reagent using a colorimeter.

Twenty-five samples of commercial American Cheddar cheese of different age and history were tested for tyramine. All were found to contain tyramine in varying degrees of concentration. The average for these samples was 384 γ per g. or 0.0384 per cent; the highest concentration was 1,199 γ per g. or 0.1199 per cent, and the lowest was 25 γ per g., or 0.0025 per cent.

A large number of miscellaneous varieties of ripened types of commercial cheese were tested for tyramine. Again, all samples were found to contain tyramine in varying concentrations. The largest amount was found in a Liederkrantz cheese which had a concentration of 1,683 γ per g., or 0.1683 per cent, while the smallest amount was found in a Roquefort cheese with a concentration of 48 γ per g. or 0.0048 per cent. The analyses were not sufficiently extensive to establish differences due to cheese variety.

Tyramine was isolated from a sample of Cheddar cheese by this method, purified, and identified as the dibenzoyl derivative.

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THE RELATIONSHIP OF THE AMOUNT OF TYRAMINE AND THE NUMBERS OF *STREPTOCOCCUS FAECALIS* TO THE INTENSITY OF FLAVOR IN AMERICAN CHEDDAR CHEESE¹

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The three previous papers in this series (3, 7, 8) have shown that the flavor of experimental American Cheddar cheese was intensified by *Streptococcus faecalis* starter, that this bacterium grew and survived in large numbers in cheese, and that tyramine, a product of growth of *S. faecalis* in the presence of tyrosine, was found in all varieties of cheese that were analyzed. Problems naturally arose concerning the effect of the presence of *S. faecalis* on the production of tyramine in cheese and any relationship that might exist between the tyramine content of commercial American Cheddar cheese and the intensity of the Cheddar flavor.

EXPERIMENTAL PROCEDURE

When the cheese from earlier experiments (3) was 5 months old, it was analyzed for tyramine. The flavor intensity of this cheese had been recorded as scored, but the scoring occurred on various days and the samples were not compared together as a lot at any time. Hence, some variation in flavor ratings must be expected due to the personal factor. The obvious relationship of tyramine to flavor intensity prompted further study.

Samples of good commercial Cheddar cheese were selected by several cheese companies to give flavor of varying intensity. These samples came from Wisconsin, Illinois and Missouri. A few samples were selected by one of the authors at factories in New York State. One sample of cheese made at Cornell University from raw milk was used; this sample was 10 years old. After all of the samples were on hand, they were arranged in order of intensity of flavor by three experienced judges. In several instances, the character of the flavor was such that it was difficult to place the cheese exactly. For example, cheese S25 possessed some Swiss cheese flavor which interfered with judgment of the intensity of Cheddar flavor.

Data were obtained on the age of the cheese, and curing temperatures usually were available. The manufacturer also stated whether the cheese had been made from raw or pasteurized milk. The cheese was tested for phosphatase by the method of Sanders and Sager (9), soluble and total

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protein (2, 10), volatile acids (6), pH, moisture, salt, titratable acidity, total bacterial count by the standard plate method (1), total enterococcus count (13), and *S. faecalis* identified by characteristics given by Sherman (11). The titratable acidity was determined by weighing out 3 g. of cheese, macerating with 10 ml. warm water in a white dish, titrating with 0.1 N alkali with phenolphthalein as indicator, and calculating the results to percentage of lactic acid.

RESULTS

The experimental cheese made with lactic starter and aged 5 months showed the lowest tyramine content, averaging 38 γ per g., and the mildest Cheddar flavor (table 1). Cheese made with both commercial lactic and *S. faecalis* starters developed the highest tyramine content, 786 γ per g.,

TABLE 1

The tyramine content and intensity of flavor of experimental American Cheddar cheese made with commercial lactic and S. faecalis starters, ripened about 5 months

Cheese no.	Curing temperature	Tyramine	Flavor intensity
	(° F)	(γ /g.)	
	Commercial lactic starter		
10464	50	21	medium—
10464	60	87	medium +
10468	50	4	mild
10468	60	40	medium
	Commercial lactic and <i>S. faecalis</i> starters		
10464	50	367	medium +
10464	60	1397	sharp—
10468	50	333	medium
10468	60	1049	medium +
	<i>S. faecalis</i> starter		
10464	50	170	medium
10464	60	830	sharp—
10468	50	122	medium—
10468	60	302	medium +

and the most pronounced Cheddar flavor. The cheese with *S. faecalis* starter alone contained 356 γ of tyramine per g. Very little tyramine was produced in cheese made from pasteurized market milk with ordinary lactic starter, whereas *S. faecalis* starter produced large amounts of tyramine. It may be observed also that there is an associated action between the two starters, which results in the production of more tyramine and slightly more flavor than obtained by *S. faecalis* alone. The acceleration of tyramine production at 60° F. as compared with 50° F. may be noted, and will be considered further in a subsequent paper.

Some variation in the exact relationship was noted in individual samples, but the intensity of the flavor of Cheddar cheese increased as the tyramine content increased (fig. 1). The trend line, drawn empirically, not only shows the trend but also, with one exception, divides the figure so that all samples of cheese above the line were cured at 50° F. and those below the line at 60° F. This observation means that the rate of flavor

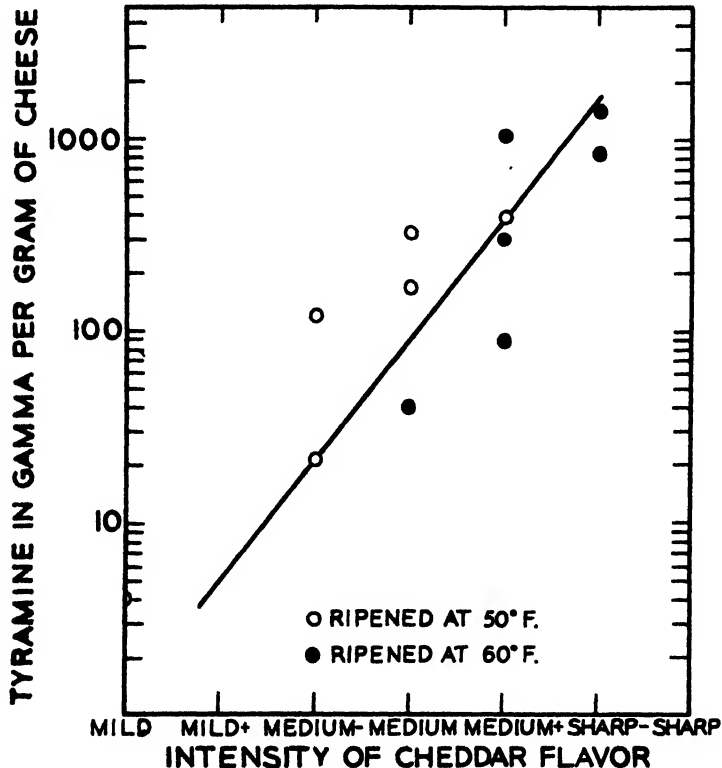


FIG. 1. The relationship between tyramine content and intensity of flavor of experimental Cheddar cheese made with commercial lactic and *S. faecalis* starters. (Ripened about 5 months.)

development at 60° F. was relatively more rapid than the increase in tyramine when compared with the rate of increases at 50° F.

The data on the 24 samples of commercial Cheddar cheese and one sample of old cheese made at Cornell University are presented in tables 2 and 3. The cheeses were grouped into units of five on the basis of flavor intensities; thus numbers 1 to 5 included the five sharpest flavored cheese in the experiment (table 4). There were fifteen raw milk cheese, six made from pasteurized milk and four were underpasteurized (table 2).

TABLE 2
The age, acidity, composition and flavor intensity of commercial American Cheddar cheese made from raw and pasteurized milk

Cheese no.	Age in months	Pasteurization		pH	Titratable acidity as % lactic	Water (%)	Salt (%)	Order of flavor intensity
		Mfg. report	Phosphatase tests (units/0.25 g.)					
G 22	120	raw	40	5.70	4.87	37.5	2.0	1
G 21	36	raw	40	5.42	3.52	31.3	1.7	2
A 4	30	raw	40	5.49	3.36	29.8	1.7	3
M 20	24	raw	30	5.61	2.86	35.0	2.0	4
K 13	10.5	raw	40	5.35	2.87	34.5	1.4	5
K 12	9.5	raw	40	5.35	2.80	34.2	1.4	6
S 25	33	raw	30	5.35	3.42	37.5	2.0	7
St 15	8.5	raw	35	5.23	2.95	34.7	1.5	8
A 5	10.5	raw	40	5.13	2.82	34.1	2.0	9
K 3	11.5	past.	1	5.22	2.89	37.0	1.6	10
St 14	8	raw	40	5.32	2.71	35.9	1.6	11
K 11	12.5	raw	40	5.46	2.21	36.6	1.2	12
LM 9	12.5	past.	9	5.22	2.57	33.4	1.9	13
K 2	4.5	past.	2	5.15	2.68	36.1	1.5	14
M 18	2.5	raw	40	5.04	2.31	38.1	1.4	15
M 17	2.5	past.	3	5.15	2.26	37.3	1.5	16
CM 24	2.5	†	6	5.14	2.03	37.2	1.3	17
CM 23	2.5	†	5	5.10	2.18	37.5	2.0	18
LM 8	12.5	past.	2	5.27	2.41	34.3	1.8	19
K 19	2.5	raw	40	5.06	2.42	39.0	1.5	20
LM 7	14.5	past.	3	5.60	2.09	32.4	1.9	21
K 1	2.5	past.	3	5.03	2.16	37.0	1.5	22
K 10	1.5	raw	40	5.26	1.92	35.7	1.5	23
M 16	1.5	raw	40	5.15	1.91	39.0	1.6	24
A 6	6	past.	5	5.32	1.71	36.0	1.4	25

† Values of 5 or greater indicate raw milk or contamination with raw milk.

TABLE 3
The flavor intensity, volatile acidity, soluble protein, and tyramine content and *Streptococcus faecalis* in commercial American Cheddar cheese made from raw and pasteurized milk

Order of intensity	Flavor		Volatile acids	Protein		Tyramine	Bacterial counts (in thousands)		
	Intensity grade			Soluble (%)	Soluble % of total		Standard plate count	Enterococci	<i>S. faecalis</i>
			(ml. 0.1 N/100 g.)			(γ g.)	(per g.)	(per g.)	(per g.)
1	sharp	+++	173.3	12.6	57.3	2330	3,000	<100	<10
2	sharp	+++	48.8	11.7	45.2	966	56,000	22,000	1,000
3	sharp	++	34.1	10.6	40.8	1199	17,000	4,000	80
4	sharp	++	24.8	11.7	42.9	1147	30,000	18,000	17,000
5	sharp	—	37.8	8.3	31.9	847	380,000	80,000	56,000
6	sharp	—	37.4	8.0	31.4	814	200,000	91,000	82,000
7	sharp	—	39.5	12.0	44.0	460	3,000	900	300
8	medium	+	41.3	7.7	32.1	746	129,000	28,000	3,000
9	medium	+	17.5	7.4	38.5	406	125,000	39,000	8,000
10	medium	+	39.3	7.5	29.7	566	285,000	53,000	3,000
11	medium	—	37.1	5.1	20.3	377	535,000	265,000	159,000
12	medium	—	34.1	6.6	25.1	233	623,000	128,000	58,000
13	medium	—	20.9	7.2	30.0	112	7,000	<100	<10
14	medium	—	40.9	6.4	25.2	177	20,000	13,000	1,000
15	medium	—	35.2	4.7	20.8	230	106,000	98,000	10,000
16	mild	+	32.2	5.1	20.8	147	55,000	29,000	<1,000
17	mild	+	22.6	4.3	19.6	123	86,000	27,000	5,000
18	mild	+	18.7	4.5	19.9	134	145,000	52,000	3,000
19	mild	+	11.9	7.1	29.5	55	10,000	<100	<100
20	mild	—	26.7	3.6	24.6	119	450,000	73,000	18,000
21	mild	—	15.5	7.7	32.1	58	40,000	<100	<10
22	mild	—	19.9	3.9	16.1	30	6,000	3,000	<100
23	mild	—	24.9	3.1	18.5	59	50,000	21,000	19,000
24	mild	—	17.6	3.0	13.3	99	205,000	90,000	31,000
25	mild	—	16.2	4.5	18.8	25	1,000	500	<20

Although cheese must be aged to develop flavor, the relationship between age and intensity of flavor was not very exact. The cheese that was 120 months old was highest in flavor, but a 6-month-old cheese was the mildest in flavor, being less cured than some cheese only 1.5 months old (table 2). The summarized data show that the ten strongest flavored cheese were oldest, but the next 15 cheese were rather uniform in age (table 4).

It is known that the pH of cheese increases with age. Apparently variations in individual cheese exceeded the effect of aging upon pH or any possible relationship of intensity of flavor to pH (tables 2 and 4). The average pH was near 5.2, the highest flavored group averaged 5.5, and the pH of all samples varied from 5.03 to 5.70. The titratable acidity varied from 1.71 for the mildest flavored cheese to 4.87 for that with the strongest flavor.

TABLE 4

The age, acidity, soluble protein, and tyramine content of commercial American Cheddar cheese grouped in units of 5 on the basis of flavor intensity

Order of flavor intensity	Age in months	pH	Titra- table acidity	Volatile acids	% soluble protein	% of total protein that is soluble	Tyra- mine
				(ml. 0.1 N acid/100 g.)			(γ /g.)
1-5	44.1	5.51	3.49	63.7	11.0	43.6	1258
6-10	14.6	5.25	2.97	35.0	8.5	33.1	598
11-15	6.1	5.24	2.49	33.6	6.0	24.3	226
16-20	4.6	5.14	2.26	22.4	4.9	22.9	115
21-25	5.2	5.27	1.96	18.8	4.4	19.8	54

The higher the titratable acidity of cheese, the more intense the cheese flavor (table 4); even the results on individual samples of cheese were noticeably consistent. There was no striking exception to this general relationship (table 2).

No significance was attached to the moisture contents, which varied from 29.8 to 39.0 per cent, and to the salt contents, which varied from 1.2 to 2.0 per cent.

Although previous studies by the authors (3) had established no definite relationship between the intensity of flavor of Cheddar cheese and volatile acidity or water soluble protein, this work was repeated in this study. In a general way, in average grouped data, the volatile acidity and soluble protein values increase with increased flavor (table 4), but this relationship does not hold for individual samples (table 3). For example, the cheese with the fourth sharpest flavor and the cheese that was twenty-third in flavor intensity both had volatile acids per 100 g. equivalent to 24.9 ml. 0.1 N alkali. The volatile acidity ranged from 16.2 to 173.3. The soluble

protein varied from 3.0 to 12.6 per cent, and the percentage of the total protein that was water soluble varied from 13.3 to 57.3 per cent, with noticeable exceptions to a definite correlation to flavor intensity in individual samples.

The data show a definite relationship of increased tyramine content and increased flavor. In the individual samples of cheese, the strongest flavored Cheddar cheese contained 2,330 γ of tyramine per g., and the twenty-fifth cheese in flavor intensity, the mildest of all flavors, had only

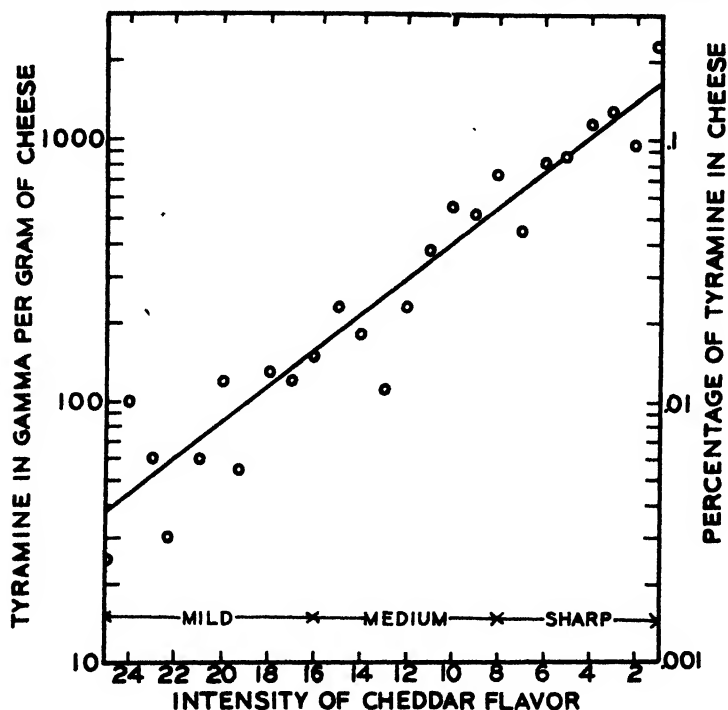


FIG. 2. The relationship between tyramine content and intensity of flavor of commercial American Cheddar cheese.

25 γ of tyramine (table 3). The tyramine ratio of these cheeses was 93 to 1. A close relationship between the tyramine content and intensity of flavor of individual samples of cheese is shown in figure 2. When one considers that there was no control over the quality of milk, method of manufacture, starter, time and temperature of ripening, and other factors of possible significance, the relationship is exceptionally good. The relationship between the tyramine content and flavor intensity was a direct semilogarithmic one. On the basis of groups of cheese as units of five in order of flavor intensity, the gamma of tyramine per gram was doubled for each group

(table 4). Tyramine added to fresh cheese curd did not give Cheddar cheese flavor and it did not aid the development of flavor during ripening.

The total bacterial counts on the cheese were made with standard tryptone-glucose-extract-milk agar, but the plates were incubated at 32° C. for 4 days to obtain maximum counts. The total bacterial counts per gram of cheese ranged from 1 million to 623 million. Counts also were made with the penicillin-azide medium of White and Sherman (13) and these counts, considered to be chiefly enterococci, ranged from less than 100,000 to 265 million. From each plate count for enterococci, 20 colonies were isolated and the number of *S. faecalis* determined, except for the possibility of confusion with *Streptococcus zymogenes*, which is not common in milk. The *S. faecalis* count was calculated from the proportion of the colonies which proved to be *S. faecalis*. The *S. faecalis* count varied from less than 20,000 to 159 million per g., and *S. faecalis* actually was isolated from 18 of the 25 samples of cheese. There appeared to be no correlation between bacterial counts and flavor intensity, and this observation was expected and in accordance with several previous publications.

DISCUSSION

It has been shown that there is a direct relationship between the tyramine content of American Cheddar cheese and the intensity of its flavor. The correlation was better for good commercial cheese selected at random than for experimental cheese made from good pasteurized market milk with special cultures and cured at different temperatures. Tyramine added to cheese curd did not give the Cheddar flavor, so tyramine is not the flavor compound. The amount of tyramine indicated the extent of activity of *S. faecalis* and probably of other bacteria, such as certain strains of the lactobacilli, which possibly may produce tyramine (4, 5). The numbers of these bacteria may increase in the early stages of cheese ripening and then decrease, so that these numbers at any one time may not be too significant.

The growth of *S. faecalis* in cheese produces Cheddar flavor, but this is only one source of flavor. That other factors are involved is indicated by the development of some Cheddar flavor in cheese with amounts of tyramine too small to indicate much growth of *S. faecalis*, and by the more rapid production of flavor than of tyramine at 60° F. when compared to 50° F. Flavor with low tyramine content always was flat, irrespective of age of the cheese.

S. faecalis and lactobacilli should be present in all raw milk Cheddar cheese, as these bacteria occur universally in raw milk. Both types of bacteria are involved in cheese ripening. *S. faecalis* is thermoduric, although survival numbers are not great, and about half of the lactobacilli

are not destroyed by pasteurization (12). Hence, pasteurized milk cheese cures slowly, as does cheese made from very low-count raw milk. The survival of *S. faecalis* and lactobacilli during pasteurization must be an important factor in present day curing of pasteurized milk cheese. The small numbers of these bacteria are increased by warmer curing temperatures.

It was reported in the first paper in this series (3) that cheese made with lactic and *S. faecalis* starters developed more flavor than cheese made with either starter alone, and that the flavor with lactic starter only was especially mild. This observation was related directly to the production of tyramine. Lactic and *S. faecalis* starters together produced more tyramine in cheese than did *S. faecalis* starter alone, even though the numbers of *S. faecalis* bacteria were greater in the cheese in which this culture alone was used, probably due to the larger inoculation in the milk. The lactic starter induced higher flavor development in cheese by its symbiotic action with *S. faecalis*.

SUMMARY

Experimental American Cheddar cheese made with commercial lactic starter from pasteurized milk developed low amounts of tyramine, 4 to 87 γ per g., and flavor of mild to medium intensity in 5 months of curing. The combination of lactic and *Streptococcus faecalis* starters in cheese produced the largest amounts of tyramine, 333 to 1,397 γ per gram, and flavor of medium to sharp intensity. *S. faecalis* starter alone in cheese produced tyramine and flavor between these two extremes.

In commercial American Cheddar cheese made from raw and pasteurized milk, cured for varying periods, there was a direct semilogarithmic relationship between tyramine content (25 to 2,330 γ per g.) and the intensity of flavor. Of the 25 cheese samples, 18 gave plate counts on the special medium of over 80,000 *S. faecalis* per g., with the high count of 159 million. The *S. faecalis* bacteria produced the tyramine, although other bacteria may contribute.

Tyramine was not the Cheddar-flavor compound, but served as a means of measuring bacterial activity that accentuated flavor production. The activity of bacteria producing tyramine did not account for all cheese flavor.

The increase in titratable acidity was related directly to cheese flavor intensity, and this relationship, even though subject to considerable variation, was too close in individual samples to be accidental. In a general way, the increase in volatile fatty acids and water soluble nitrogen was related to flavor intensity, but variations in individual samples prevented a definite correlation and also established that these changes were incidental to flavor development.

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DETERMINATION OF VITAMIN A IN MILK¹

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Milk is an important natural source of dietary vitamin A. Recent interest in the enrichment of milk has emphasized the need for a simple and reliable method of assay for control purposes. Attempts to determine preformed vitamin A in milk in this laboratory by several methods found in the literature have been unsuccessful. These procedures, although reliable for other types of products, gave low recoveries (approximately 80 per cent of the theoretical) of the added vitamin. In these tests the milk was enriched with a vitamin A emulsion similar in composition to milk itself.² The low values may be attributed to a combination of causes, including destruction of the vitamin during hot saponification, inadequate cold saponification, loss due to adsorption on the milk protein, inefficient extraction, or failure to take into account the effect of inhibitors in the antimony trichloride reaction.

In the present study, vitamin A in milk is determined quantitatively by a method which involves cold saponification with potassium hydroxide, extraction of the vitamin with diethyl ether, evaporation of the solvent, and solution of the residue in chloroform.³ The vitamin then is allowed to react with antimony trichloride and the resulting blue color measured in an Evelyn photoelectric colorimeter. The effect of compounds which inhibit the color formation is evaluated by means of an internal standard.

The importance of adequate, cold saponification in the analysis of milk for vitamin A has been recognized by other investigators (1). The use of the internal standard has been described (5) and has received considerable attention in the development of analytical procedures for the determination of vitamin A in enriched margarine.

METHOD

Reagents

Aldehyde-free alcohol. Reflux 1 l. of 95 per cent ethyl alcohol with 10 g. of potassium hydroxide on a steam bath for 5 hours and then cool. Add 0.5 g. granulated aluminum and distill on a steam bath. Prepare fresh each month.

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² The concentrate used for enrichment consisted of a butter oil solution of vitamin A, homogenized with water and skim milk solids.

³ The saponification, extraction, evaporation, and washing steps are essentially those employed in the laboratory of the Wisconsin Alumni Research Foundation. The details of the method were kindly supplied by Dr. Carl H. Krieger.

Sixty per cent potassium hydroxide solution. Dissolve 60 g. of potassium hydroxide in 40 ml. of distilled water.

Antimony trichloride reagent. Grind 250 g. of antimony trichloride with 250 g. of anhydrous sodium sulfate. Suspend the mixture in 1,000 ml. of freshly redistilled chloroform, stir mechanically for 15 minutes, filter through rapid paper, and store in the dark in a glass-stoppered amber bottle. The reagent is stable for at least 1 month. If turbid, filter again immediately before use.

Saturated sodium chloride solution. Suspend 800 g. of sodium chloride in 2,000 ml. of water, stir mechanically for 15 minutes and allow to stand overnight to assure saturation.

Ethyl ether. Distill U.S.P. ethyl ether freshly each day.

Chloroform. Distill C.P. grade chloroform freshly each day discarding the first 10 per cent of the distillate.*

Vitamin A standard. Dissolve 100 mg. of U.S.P. vitamin A Reference Standard (10,000 U.S.P. units per g.) in 100 ml. of freshly redistilled chloroform. This solution contains 10 U.S.P. units of vitamin A per ml. and is prepared fresh each day.

Acetic anhydride. Use C.P. grade.

Special Apparatus

Separatory funnels. Three 500-ml. pear-shaped separatory funnels are required.

Evelyn photoelectric colorimeter (macro) with matched colorimeter tubes. This instrument is manufactured by the Rubicon Company, Philadelphia, Pennsylvania.

Rapid delivery pipette. This may be prepared by cutting the tip from a 10-ml. volumetric pipette, leaving an opening about 2 mm. in diameter. The pipette need not be recalibrated.

Procedure

To 100 ml. of milk in an amber Erlenmeyer flask are added 50 ml. of aldehyde-free alcohol and 10 ml. of 60 per cent potassium hydroxide. The suspension is mixed and allowed to stand overnight in a dark cabinet. In the morning the sample is transferred to a 500-ml. amber separatory funnel. The flask is washed consecutively with 40-, 40-, and 20-ml. portions of distilled water and once with 100 ml. of redistilled ether. The washings are added to the separatory funnel. The latter is shaken thoroughly and then allowed to stand until a sharp separation of the phases is

*Occasionally samples of chloroform are obtained which are unsatisfactory as solvents for vitamin A. The vitamin standard solution in chloroform should show less than 5% decomposition when stored for 24 hours in the dark.

observed. The lower layer is drawn off into a second separatory funnel. This is shaken with 75 ml. of ether. After the phases have separated, the lower (aqueous) layer is drawn off into a third separatory funnel. The ether layer is added to the extract in the first funnel, along with a 25-ml. ether wash. The aqueous phase is re-extracted twice more with 75- and 50-ml. portions of ether, respectively. The ether phases all are combined in the first separatory funnel and are washed four times with 100-ml. portions of distilled water. To prevent the formation of emulsions, the first two washings merely are poured through the ether, without shaking, and then drawn off. The funnel is shaken gently during the third washing and vigorously during the fourth. Sufficient time for complete separation of the phases must be allowed at all times. Finally, the ether extract is washed twice with 75-ml. portions of saturated sodium chloride solution, shaking vigorously each time. The last salt solution is separated sharply from the ether phase.

The ether extract is transferred to a 500-ml. amber distilling flask containing two or three glass beads. The separatory funnel is rinsed with 25 ml. of redistilled ether and the wash added to the flask. The ether is removed by distillation on a steam bath until approximately 5 ml. remain in the flask. The remainder is evaporated off at room temperature with the aid of a stream of carbon dioxide. While still under the inert atmosphere, the vitamin A in the residue is dissolved immediately in sufficient chloroform to produce a concentration of approximately 10 U.S.P. units per ml.

Into a series of labeled Evelyn colorimeter tubes in a wooden rack are pipetted 2 ml. of chloroform solvent (tube *A*), 1 ml. of chloroform extract of sample + 1 ml. of chloroform solvent (tube *B*), and 1 ml. of chloroform extract of sample + 1 ml. of chloroform solution of vitamin A standard (tube *C*).

To tube *A*, one drop of acetic anhydride is added, followed by 10 ml. of antimony trichloride reagent, the latter from the rapid delivery pipette.⁵ The colorimeter, containing a 620 m μ filter, then is set at 100 per cent transmission with the solution in tube *A*. The tube is removed and the "center setting" noted. The latter is employed to reset the instrument before each subsequent reading.

Tube *B* is placed in the instrument, one drop of acetic anhydride is added, and 10 ml. of antimony trichloride reagent are added rapidly. The galvanometer needle first fluctuates rapidly, "pauses" for a second or two, then drifts slowly as the blue color fades. The per cent transmission at the pause point is recorded. Tube *C* is measured similarly.

⁵ Because antimony trichloride solution is extremely sensitive to moisture and is corrosive, it must be pipetted with a rubber bulb, not by mouth.

Because of difficulty in observing the pause point and in order to obtain reliable results, tubes *B* and *C* are set up and measured in triplicate, and the average per cent transmission of each determined. The latter (*G*) is converted to photometric density ($L = 2 - \log G$), employing the chart provided with the instrument. The vitamin A content of the milk sample is calculated employing the formula:

$$\frac{L_B}{L_C - L_B} \times 10 \times \frac{V}{100} \times 946 = \text{U.S.P. units of vitamin per quart.}$$

(*V* = cc. of chloroform solution of unsaponifiable extract)

EXPERIMENTAL RESULTS AND DISCUSSION

The colorimetric method as described by Oser *et al.* (5) has been employed successfully in these and other laboratories for the determination

TABLE 1

Recovery tests of vitamin A added to milk, employing previously published procedures (1, 5)^a

Procedure	Milk sample	Vitamin A added	Total vitamin A found	Vitamin A recovered	
		(USP units per qt.)			(%)
Boyer <i>et al.</i> (1)	Jan. milk, homogenized	0	960		
		2000	2560	1600	80
		4000	3970	3010	75
		6000	6190	5230	87
	Oct. milk, cream-line	0	1020		
		4000	4270	3250	81
Oser <i>et al.</i> (5)	Oct. milk, homogenized	0	1510		
		4000	4800	3290	83
	Oct. milk, homogenized	0	1420		
		4000	4700	3280	82
	Oct. milk, cream-line	0	1320		
		4000	4580	3260	82
	Oct. milk, homogenized	0	1200		
		4000	4400	3200	80

^a For each recovery test, the vitamin was added directly to the milk immediately before the analysis and the enriched sample was carried through the entire determination. The concentrate employed for enrichment consisted of a solution of vitamin A esters in butter oil, homogenized with water and skim milk solids.

of vitamin A in a wide variety of food products. Boyer *et al.* (1) have applied the antimony trichloride reaction to the analysis of milk. The recent interest in the enrichment of milk with vitamin A prompted an examination of the reliability of these procedures. The results of these tests are presented in table 1. Milk samples were enriched with vitamin A at the levels indicated, employing a concentrate similar in composition to milk itself. This concentrate consisted of a solution of vitamin A

esters in butter oil, homogenized with water and skim milk solids, and contained 30,000 U.S.P. units per ml. The blank and enriched samples were analyzed immediately according to the published procedures. Approximately 80 per cent of the added vitamin was recovered in each case. Because of the physical nature of milk, the complete extraction of its fat-soluble constituents is particularly difficult. Therefore, it is not surprising that the determination of vitamin A in milk requires a special technique.

Similar recovery tests, employing the procedure described in the present

TABLE 2

Recovery tests of vitamin A added to milk employing present procedures

Milk sample	Vitamin A added	Total vitamin A found	Vitamin A recovered	
	<i>(USP units per qt.)</i>			<i>(%)</i>
	0	1310		
<i>A</i>	2000	3270	1960	98
Oct. milk,	4000	5500	4190	105
homogenized	6000	6970	5660	94
<i>B</i>				
Oct. milk,	0	1200		
cream-line	4000	5250	4050	101
<i>C</i>				
Oct. milk,	0	1070		
cream-line	4000	4970	3900	98
<i>D</i>				
Jan. milk,	0	830		
homogenized	4000	4830	4000	100
<i>E</i>				
Jan. milk,	0	920		
cream-line	4000	4950	4030	101

^a For each recovery test, the vitamin was added directly to the milk immediately before the analysis and the enriched sample was carried through the entire determination. The concentrate employed for enrichment consisted of a solution of vitamin A esters in butter oil, homogenized with water and skim milk solids.

paper, are reported in table 2. Recovery values ranged from 94 to 105 per cent, with an average of 99.6 per cent.

The use of an internal standard in the determination of vitamin A decreases the precision of the analysis. Therefore, some investigators have employed a reference curve, obtained with pure solutions, to calculate their results or, since the reaction obeys Beer's law, an external standard of 10 U.S.P. units. The data employed in calculating the results reported in table 2 demonstrate the importance of the internal standard or increment. These data are presented in table 3. In a given test extract, the photometric density has been found to be proportional to the vitamin A content. However, biological materials contain substances which inhibit

the reaction, and the constant of proportionality is not the same in all extracts. Thus in sample A, the increments due to 10 units of added vitamin A were 0.184, 0.174, 0.173 and 0.180, respectively, in the four solutions tested. Employing the same reagents at the same time, 10 units of vitamin A in pure chloroform gave a photometric density of 0.201. Thus, the use of a reference curve in calculating the vitamin A content of the above samples would give values erroneously low by approximately 13 per cent. Samples B and C likewise required the internal standard. On the other hand, either method of calculation could be employed with D and E. The

TABLE 3
Importance of the internal standard

Milk sample	Vitamin A added	Photometric density			
		Tube B	Tube C	Internal standard ^a	External standard ^b
	(USP units per qt.)				
A	0	0.102	0.286	0.184	0.201
	2000	0.240	0.414	0.174	0.201
	4000	0.201	0.374	0.173	0.201
	6000	0.177	0.357	0.180	0.201
B	0	0.047	0.233	0.186	0.199
	4000	0.184	0.362	0.178	0.199
C	0	0.043	0.233	0.190	0.204
	4000	0.182	0.364	0.182	0.204
D	0	0.036	0.240	0.204	0.204
	4000	0.189	0.387	0.198	0.204
E	0	0.039	0.238	0.199	0.204
	4000	0.184	0.387	0.203	0.204

^a Photometric density due to the reaction of antimony trichloride with 10 USP units of vitamin A added to the chloroform solution of the unsaponifiable extract of the milk sample.

^b Photometric density due to the reaction of antimony trichloride with 10 USP units of vitamin A in chloroform.

importance of the internal standard also has been established in the determination of niacin (4) and pyridoxine (3).

If the total vitamin A content of unenriched milk is of interest, β -carotene should be determined in the extract by carefully evaporating off the ethyl ether, taking up the residue in petroleum ether, and fractionating the pigments with diacetone alcohol (2). As much as one-fourth of the total vitamin A potency of unenriched milk is due to carotene, although in the case of summer milk from Guernsey cows, almost 50 per cent may be present as the provitamin (1). In milk enriched with preformed vitamin A, these proportions are considerably smaller.

Though carotene reacts with antimony trichloride to form a blue pigment, this does not interfere appreciably in the determination of the preformed vitamin because of differences in the rates and sensitivities of the two reactions. The photometric density at 620 $m\mu$ due to one U.S.P. unit of β -carotene is only one-twelfth that due to one U.S.P. unit of preformed vitamin A 4 seconds after the addition of antimony trichloride reagent (5).

SUMMARY

1. Vitamin A in milk was determined quantitatively by cold saponification with potassium hydroxide, extraction with diethyl ether, evaporation of the solvent, and solution of the residue in chloroform. The blue color formed by reaction with antimony trichloride was measured in a photoelectric colorimeter.

2. Theoretical recoveries of added vitamin A were obtained. The concentrate employed was a solution of vitamin A in butter oil, homogenized with water and skim milk solids. Two other procedures for the determination of vitamin A gave recoveries of only 80 per cent.

3. Colorimetric evaluation of the vitamin A content of the final extract included the use of an internal standard. Calculations based upon a reference curve obtained in pure solutions gave low recovery values in some samples.

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THE EFFECT OF CLIPPING THE UDDERS OF COWS ON THE QUALITY OF MILK

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The effect of clipping of udders of cows upon the quality of milk has been the subject of original study of but a few investigators (2, 3). The information available, though brief, indicates benefit from the practice. Among milk producers and quality supervisors there seems to be varied opinion as to the benefit of the clipping of udders. In view of the increasing emphasis on the methods for obtaining milk of good quality, a study was made of the effect of clipping of udders and adjacent areas on certain quality properties of milk.

EXPERIMENTAL PROCEDURE

Handling of Cows. The experiment was divided into four periods as follows: (a) Control period, milking by machine. November 20 to December 2, 1946. (b) Second period, milking by machine. December 3, 1946, to January 30, 1947. (c) Third period, milking by hand. February 6 to March 26, 1947. (d) Fourth period, milking by machine. March 26 to April 4, 1947. In the control period, prior to clipping any of the animals, the numbers of bacteria in the milk of the individual cows milked by machine were determined. For the second period, alternate cows, as they stood in line, were clipped. The animals were reclipped during the third period. The clipped area was posterior to a line from the pinbones to the navel, including thighs, flanks, and udder, and the tail except for the switch. The area clipped is illustrated in figure 1. As they freshened and were introduced in the milking line, alternate cows were clipped.

The conditions in the barn were comparable to those usually found in a city fluid milk area. Wood shavings were used liberally for bedding, but not in excess. The cows were groomed once daily, usually during the morning but not immediately before the milkings. The night's accumulation of manure was in the gutters at the time of the morning milking. The cows seldom were soiled at milking time to a degree greater than that

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of having loose bedding and dirt clinging to the body. The herd was kept indoors without access to an exercise yard.

Treatment of milking utensils. All utensils used in this experiment were new. Prior to each period of use, four disassembled milking machine units, excluding the pails, were sterilized in a steam autoclave. The stainless steel machine pails and milk cans were jet steamed for a period of 3 minutes, then filled with a solution of 200 p.p.m. available chlorine for 1 hour, drained, and covered with parchment paper. The units then were assembled and immersed in a solution containing 200 p.p.m. of chlorine. Two gallons of sterile distilled water added in three portions was used to rinse the chlorine from one of the machine pails chosen at random. The



FIG. 1. One of the cows used in the experiment, showing the area clipped.

machine head then was adjusted and another 2 gallons of sterile distilled water drawn through the teat cup assembly and into the pail. The average bacteria count per ml. of the rinse water was ascertained to be 2 in the evening and 1 in the morning tests.

Just before milking, the udder of each cow was wiped with an individual cloth previously immersed in a warm solution of 200 p.p.m. available chlorine. The cows were fore-milked into a strip cup, and this milk was discarded. The sterilized machine unit was attached with special care to avoid contamination. The cows were machine stripped. The intact milking unit was taken immediately into the milk house for sampling and weighing the milk. The milk then was carefully transferred to identified sterile covered 10-gallon cans. The milk was not strained. The machine

was partially dismantled and all parts which came in contact with milk were rinsed twice in warm tap water, followed by an immersion rinse in a solution containing 200 p.p.m. available chlorine. The reassembled unit then was returned for immediate use on other animals. Terminating each milking period, the machines were water rinsed, completely disassembled, washed and scrubbed, rinsed in hot water, and stored on racks for later milking periods.

For hand milking, open-top oval dairy pails were sterilized, as were the milking machine pails. The cows' udders were wiped in the same manner as when machine milked. The six milkers immersed their hands in a solution containing 200 p.p.m. chlorine prior to each milking, after which they touched nothing except the cows' teats until each milking assignment was completed. The milking stools were handled for the milkers by assistants. To obtain balanced results, the milkers alternated at random between clipped and non-clipped cows. The milk obtained by hand milking of each cow was immediately transferred to the milk house for sampling.

Milk sampling procedures. The following procedures were used for bacteriological analysis of the milk: (a) A sample from each pail of milk from each cow was obtained immediately after it was conveyed to the milk house. (b) Separate composite samples of the milk from the clipped and non-clipped cows, respectively, were taken immediately from each 10-gallon can filled during the milkings. (c) Separate composite samples and off-the-bottom sediment tests of the milk from the clipped and non-clipped cows were taken from each can at the time the milk was delivered to the dairy plant.

The samples of milk, transferred by means of a sterile glass tube thief to sterile screw cap bottles, immediately were placed in ice water and so kept until the bacteriological tests were undertaken, regularly within 60 minutes. The filled milk cans were stored in a water immersion refrigerator at 33 and 34° F. The milk in cans was held until approximately 7:00 a.m., when it was trucked to the dairy plant, a distance of approximately 0.25 mile (milkings were begun at 3:30 a.m. and p.m.).

Methods of testing. Standard bacteria plate count estimates of the milk were made as described in Standard Methods for the Examination of Dairy Products, 8th Edition (1). The raw milk samples were plated in duplicate at dilutions of one in ten. Samples pasteurized in the laboratory were plated without dilution. The milk was pasteurized at 143° F. in a thermo controlled bath for 30 minutes, using 5-ml. portions and a blank open tube for thermometer observation. All counts are reported as bacteria per ml.

The presence of extraneous material in the milk was determined by use of a Langsenkamp-Wheeler off-the-bottom sediment tester which withdrew a 1-pint sample from each 10-gallon can of composited milk.

RESULTS

In table 1 are presented the bacteria counts of the milk (weighted arithmetic averages) from those cows which were milked throughout the first and the second periods. In the first or control period, all udders were unclipped; in the second period, udders of alternate cows were clipped. The average count per ml. of the milk from those cows not clipped was determined as 1,308 in the control period and 1,869 per ml. in the second

TABLE 1

Weighted arithmetic average count per ml. of samples of milk taken from individual milkings obtained by machine during preliminary period and after clipping part of cows

Treatment after preliminary period	No. of cows	Prior to clipping any cows		After clipping part of the cows	
		No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
Unclipped	10	19	1308	84	1869
Clipped	13	22	1629	92	1397

period. The count per ml. of the milk from cows subsequently clipped decreased from the control period average of 1,629 to 1,397. Since, during the second period, the average count of milk from the cows remaining unclipped increased, while that of the milk from the cows clipped decreased, the apparent over-all difference in numbers of organisms in the milk appears to indicate beneficial effects of clipping.

In table 2 are presented the weighted arithmetic averages of the counts per ml. of milk from cows during the second period, when milked by machine, and during the third period, when milked by hand. The results are presented on the basis of both morning and evening milkings. The number of cows involved in this analysis varied due to drying-off and

TABLE 2

Weighted arithmetic average count per ml. of samples of milk taken from individual milkings

Time of milking	Clipped			Unclipped		
	No. of cows	No. of samples	Weighted arithmetic av. count per ml.	No. of cows	No. of samples	Weighted arithmetic av. count per ml.
<i>Machine milked</i>						
Evening ..	13	92	1548	23	115	1805
Morning	13	93	1375	16	101	1317
<i>Hand milked</i>						
Evening	14	83	877	16	83	1484
Morning ...	14	102	830	16	104	1143

TABLE 3

Weighted arithmetic count per ml. of composite samples of milk from clipped and unclipped groups of cows

Time of milking	Clipped		Unclipped	
	No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
<i>Machine milked</i>				
Evening	8	1590	8	2381
Morning	8	1254	8	1245
<i>Hand milked</i>				
Evening	6	566	7	1250
Morning	8	771	8	1000

freshening. In addition to these natural causes, some samples were omitted because of explainable contamination, such as dropping of teat cups into the bedding, cows kicking into milk pails during hand milking, and sudden evidence of mastitis. The results show that the average count per ml. of the milk obtained by machine milking from clipped cows differed but slightly (4.4 per cent more in morning and 2.4 per cent less in evening) from that similarly obtained from unclipped cows. On the other hand, the results show that the average counts of the milk obtained by hand milking from clipped cows were less (40.9 per cent for morning and 28.0 per cent for evening) than those similarly obtained from unclipped cows.

In table 3 are presented the average counts per ml. of the composite samples of milk obtained from filled 10-gallon cans. The average count per ml. of this milk from the clipped cows was definitely less than that from the non-clipped cows. The over-all difference approximated 30 per cent. A similar relationship in bacteria numbers of the evening's milk refrigerated for approximately 14 hours before being sampled also was

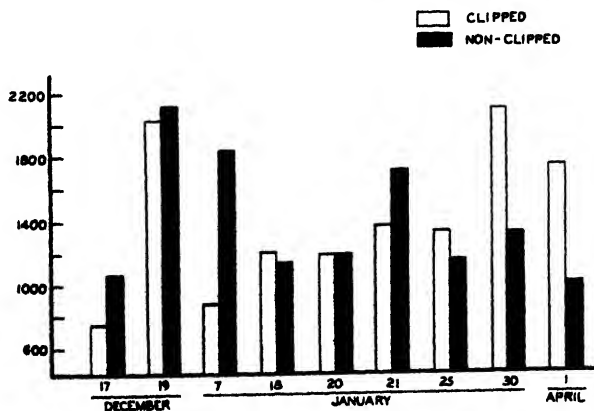


FIG. 2. Average bacteria count of individual milkings of cows milked by machine in morning.

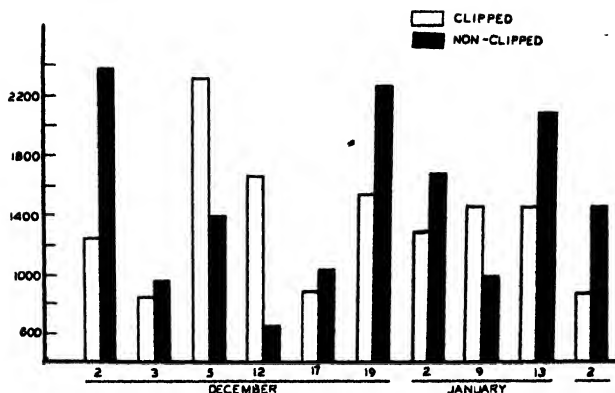


FIG. 3. Average bacteria count of individual milkings of cows milked by machine in evening.

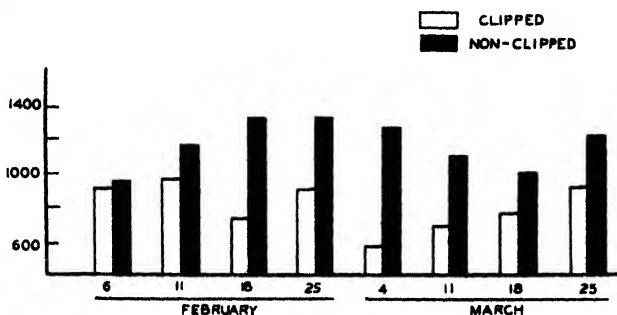


FIG. 4. Average bacteria count of individual milkings of cows milked by hand in morning.

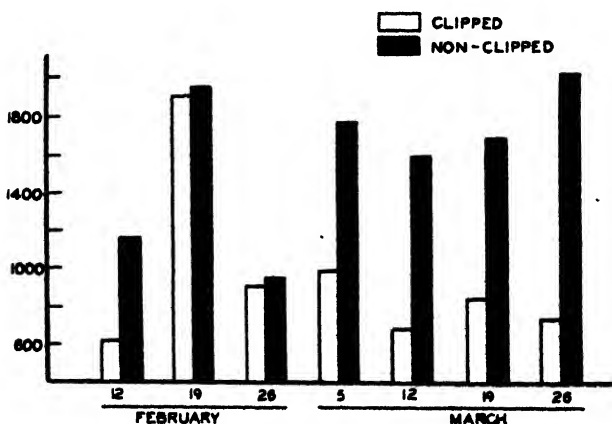


FIG. 5. Average bacteria count of individual milkings of cows milked by hand in evening.

observed. The counts of the composite samples subsequently pasteurized ranged from 3 to 13 per ml. and were so low that no significance could be attached to the figures.

The average counts per ml. of the milkings from the clipped and non-clipped cows, during the morning and evening milking, for each milking period included in the study, are presented in figures 2, 3, 4 and 5. An analysis of variance of the data presented in the graphs shows there is a significant difference in the count per ml. of the milks obtained by hand milking between the clipped and non-clipped cows. The difference in the counts per ml. of the milks obtained by machine milking from clipped and non-clipped cows was not statistically significant.

The tests for extraneous material present in the milks were conducted at the time the milks were delivered to the platform of the plant. No

TABLE 4
*Frequency of the grades (Wisconsin Standards) of the tests
for extraneous material in milk*

	Grades			
	1	2	3	4
<i>Machine milking</i>				
Clipped cows	1	16	7	1
Unclipped cows		14	10	0
<i>Hand milking</i>				
Clipped cows			8	20
Unclipped cows			5	18

difference in milks from clipped or unclipped cows could be determined. However, the amount of extraneous material in the milk obtained by hand milking was much greater than in that obtained by machine. The summary of the tests is tabulated in table 4.

The udders usually were clean prior to washing except for loose dirt or shavings clinging to the hair. The time spent in washing the udders prior to milking did not differ appreciably between the clipped and unclipped cows. This was used as the routine stimulus for let-down of milk, and the time spent was more than adequate to cleanse the teats and udder of all visible dirt.

DISCUSSION AND SUMMARY

The effect of the clipping of cows upon the quality of milk was determined by colony plate count and tests for presence of extraneous material. The clipping tended to lower the average bacteria counts per ml. of the milk, whether the milking was done by machine or by hand. The average counts per ml. of the milk obtained by machine from clipped and unclipped cows were 3,042 and 3,458, and by hand 1,643 and 2,996, respectively. The advantages of clipping were statistically significant for the milks obtained by hand milking.

The average counts per ml. of the milk obtained by machine were greater than those of the milk obtained by hand. This might be due to the ends of the teats being bathed to some extent by milk during machine milking, resulting in rinsing of organisms into the milk. When the milking is performed by hand, the bathing action does not occur. Although the average bacteria count per ml. of the milk obtained by machine was greater than when obtained by hand, the amount of extraneous material present was observed to be greater in the milk obtained by hand. The clipping of cows caused no measurable difference in the amount of extraneous material in milk when obtained by either machine or hand milking.

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STORAGE AND TREATMENT OF MILKING MACHINE INFLATIONS UNDER FARM CONDITIONS^{1,2}

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Difficulty in producing high quality milk frequently has been attributed to using milking machines that were not in the proper state of sanitation. Many procedures have been advocated as a means of sanitizing the rubber inflations and tubing. Most of these procedures were discussed in a previous article (3) in which the authors presented results of a laboratory study of rubber inflations for milking machines. The study reported: (a) the extent of fat absorption, (b) the extent of storage solution absorption, (c) the deteriorating effect of inorganic chlorine, and (d) the advantage of boiling rubber parts in lye solution at intervals as a means of saponifying entrained fat, thus improving their sanitary condition.

Mallman *et al.* (4) recently reported a study in which producers used a variety of sanitizers. This study indicated that cationic germicides were more effective as sanitizing agents than lye and chlorine, as measured by lower total and thermoturcic counts of milk.

Dahlberg (2) also recently reported results of milking machine sanitizing studies and found high bacterial counts with dry-stored inflations and low counts and clean tubes with lye solution on rack storage. He reported that dry storage following washing and rinsing with cationic germicides was not satisfactory.

Investigation seemed desirable to determine certain storage and treatment practices under practical farm conditions. To this end farm studies were made over an extended period on the treatment and storage of rubber inflations and tubing of milking machines, studying the physical and bacteriological cleanliness of the rubber and the bacterial population of milk produced through their use.

EXPERIMENTAL PROCEDURE

Preliminary observation of producer methods. Producers of milk from one dairy plant were used for this study over an 18-month period. The early portion of the study consisted of making inspection of milking machines for cleanliness and method employed for storage between use, while weekly bacterial counts were made on milk from each producer.

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During this period certain storage practices were found to give satisfactory results and others were observed to have some objectionable features that deemed them undesirable for rubber storage. The producers having generally satisfactory results were those using lye and cationic germicide solutions, and dry storage following scalding with very hot water. Acid solution had no appreciable germicidal property and inorganic chlorine caused gross deterioration of rubber. This portion of the study was continued by making bacterial counts of sterile water rinses of the inspected rubber parts and bacterial counts of the milk produced through their use.

Based on these observations, further study was made comparing dry, lye and cationic solution storage under conditions wherein the washing procedure employed before their use would be the same. Producers operating two milking machines each were chosen for this study. Four different storage treatments were compared: (a) dry storage following the regular washing procedure, (b) dry storage following washing and rinsing, after which a subsequent rinse with 1 gallon of 200 p.p.m. cationic germicide solution was used, (c) washing followed by solution storage, 200 p.p.m. cationic germicide, and (d) solution storage with 0.5 per cent lye solution. All milking machines were washed using a washing powder consisting of anionic synthetic detergent and near-neutral polyphosphate. Long tube milkers generally were washed by a flush washing procedure, while short tube milkers generally were washed disassembled.

Each producer having two milker units was asked to follow different designated storage procedures on each unit as a means of comparing the sanitary condition of the inflations and tubing affected thereby, thus eliminating the factor of washing effectiveness, which was assumed to be the same for both units. Examinations and bacterial counts were made over a period of 5 to 10 weeks, the greatest number involving ten weekly examinations.

Bacterial counts of inflations were made from water rinses, using 500 ml. of sterilized water, chilled by icing to ice water temperature. This water was emptied into the cups and tubing of long tube milkers while supporting these parts in an upright position. After filling, the water was returned to the water jar by discharging through the rubber tubing. Short tube milker inflations were filled after pinching shut the tubing near the outlet. Four inflations from each short tub milker unit were rinsed similarly. The samples again were placed in iced water and within 4 hours were plated for total count, using standard methods (1). Thermoturic counts of rinse water were made by plating the water after laboratory pasteurization at 143° F. for 30 minutes.

Weigh-can samples of each producer's milk supply were taken the day after examining inflations. These were plated for total and thermoturic count. Examination for physical cleanliness of inflations and tubing was

made by scraping the inner lining of inflations with a spatula and rodding the tubing. Evidence of soiling was noted.

RESULTS

Use of dry storage with and without cationic germicide rinse. Bacterial counts resulting from dry storage as obtained from three producers are shown in table 1. A survey of bacterial counts of inflations shows no important improvement in sanitation when 200 p.p.m. cationic germicide solutions were used for rinsing following washing. This was true when high bacterial counts were obtained with producers 3L and 17L, as well as when low counts were secured by producer 36L, whose low counts were attributed to rinsing the washed utensils with a liberal quantity of very

TABLE 1
Influence of cationic rinse on bacterial population of rubber inflations

Producer	Method of storage ^a	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermiduric	Total	Thermiduric
36L ^b	A	10	5,000	1,300	25,000	600
	B	10	3,000	1,500	100,000	600
	C	10	4,000	1,200		
3L	A	11	550,000	4,600	40,000	1,300
	B	9	220,000	2,100	46,000	500
	C	9	650,000	1,600		
17L	B	5	2,800,000	5,600		
	C	5	1,400,000	5,000	160,000	12,000

^a A = dry, preliminary trials; B = dry; C = dry, cationic germicide rinse.

^b L = long tube milker.

hot water. Total and thermiduric counts of inflation rinses from producers 3L and 17L were excessively high, and the cationic rinse gave insufficient germicidal property by this method of application.

Total and thermiduric counts of milk produced with the inflations also are shown in the table. No attempt was made to relate these counts to the particular sanitizing treatment applied to inflation assemblies, since it was not practical to segregate milk produced by each milker unit. Normally low thermiduric counts were obtained from producers 36L and 3L, while those of 17L were high, as were also the total counts of inflations from this producer. Averages obscure the conditions that were involved in this producer's counts. These conditions are presented in detail in table 5.

Dry storage versus storage in cationic solution. Bacterial counts of rubber inflations and tubing secured when comparing dry storage following washing with solution storage using 200 p.p.m. cationic germicide solution are shown in table 2.

Three producers' machines were observed. The most pronounced difference in results was secured with producer 1S, for whom cationic solution storage results were very satisfactory, with an average count of 11,000 for the nine trials. The dry storage inflations, while similarly washed, were excessively high in count, averaging 3,800,000 for the nine trials. The extreme difference secured with this producer is accounted for by the observation that washing and rinsing were done with medium warm water and no attempt was made to sanitize the dry storage inflations.

Producers 2S and 26L had low bacterial counts on both dry and cationic solution trials, counts that were markedly lower than in the preceding preliminary trials. Both producers were habitually careful about cleaning their milkers and were discovered to be somewhat reluctant to

TABLE 2
Influence of cationic solution storage on bacterial population of rubber inflations

Producer	Method of storage ^a	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermogenic	Total	Thermogenic
1S ^b	A	9	170,000	3,100	39,000	700
	B	9	3,800,000	4,700	250,000	5,200
	C	9	11,000	1,700		
2S	A	7	30,000	3,000	35,000	800
	B	10	2,500	1,000	11,000	500
	C	10	3,500	2,300		
26L	A	7	58,000	1,900	230,000	1,300
	B	6	1,300	700	830,000	900
	C	6	500	500		

^a A = dry, preliminary trials; B = dry; C = cationic solution.

^b S = short tube milker; L = long tube milker.

use sanitizing solution storage. They therefore were concerned about having the dry storage inflations as bacteria-free as those stored with cationic solution and used very hot water for sanitizing following washing.

All of the machines in table 2 were washed disassembled. Machines and inflations of producers 2S and 26L always were found in an excellent state of cleanliness, while those of 1S at times were criticized for being slightly slimy in the upper portion of the inflations.

The conditions of the milking machine tubing and inflations were not necessarily reflected in the total bacterial counts of the milk. High bacterial counts were obtained in milk samples from producer 26L, in spite of his use of clean and well sanitized inflations and tubing. The high counts were attributed to delayed and inadequate cooling caused by the milk house being located near the farm residence across a highway from the barn. Also, this producer had more milk than could be accommodated in his cooling tank. However, there was considerable increase in thermogenic

count on the milk in samples from producer 18 when the milker inflation total counts were high.

Trials comparing solution storage using 0.5 per cent lye and 200 p.p.m. cationic germicide are shown by bacterial counts listed in table 3. Most apparent is a reduction of bacterial count when solution storage was used, in contrast to the situation when dry storage was employed by the various producers during the preliminary trials.

When long tube milkers requiring solution racks were used for storage of inflations and tubing, as shown by producers 5L, 9L, 15L, 23L, and 59L

TABLE 3
Influence of cationic solution and lye solution storage on bacterial population of rubber inflations

Producer	Method of storage ^a	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoturic	Total	Thermoturic
5L	A	11	7,800	1,400	47,000	1,200
	B	9	2,000	800		
	C	9	2,000	1,000	48,000	6,200
9L	B	6	5,900	800		
	C	6	6,600	600	31,000	600
15L	A	11	390,000	6,000	9,600	1,000
	B	9	1,400	1,500		
	C	9	1,400	600	10,000	600
23L	A	11	140,000	3,500	100,000	
	B	8	1,100	800		
	C	8	1,300	500	83,000	900
59L	A	8	170,000	13,000		
	B	9	7,600	2,800	28,000	400
	C	9	1,100	600		
618	D ^b	11	13,000	4,000	44,000	4,700
	B	9	24,000	1,900		
	C	9	1,300	800	10,000	600

^a A = dry, preliminary trials; B = lye solution; C = cationic solution.

^b D = lye solution, preliminary trials.

lye and cationic germicide solutions were equally effective as sanitizing agents, as indicated by total bacterial counts of rinses. A consistent, though small, decrease in thermoturic bacterial counts of rinse samples was secured in favor of cationic germicide solution storage over lye solution storage. Likewise, there was a general reduction in milk thermoturic counts when solution storage was used compared with dry storage. One exception to this is observed in producer 5L. Although the counts on the inflations and tubing were low, this producer was not successful in maintaining general physical cleanliness of the rubber parts of his machines. Usually spatula scrapings from the inflations gave heavy milk sludge deposits. This condition was found to be caused by dipping milk-coated

teat cups in warm chlorine solution of 200 p.p.m. strength before milking the next cow, without previously rinsing off the milk in cold water. Such a treatment caused a slimy film that was not removed readily during washing.

When inflations were stored in solution jars, as was required of the short tube inflations of producer 61S, cationic solution storage was more effective than lye as a sanitizing agent. The inflations stored in the cationic solution yielded an average rinse count of 1,300 in contrast to 24,000 for lye storage.

Higher rinse counts were secured using lye in jar storage that were obtained with lye used in solution racks. This was considered to be due to repeated use of the same lye solution for a 7-day period in jar storage, whereas a fresh lye solution was applied between each milking when rack storage was used.

Physical cleanliness and bacterial cleanliness. A tabulation of the

TABLE 4

The relationship between bacterial count and physical cleanliness of milker inflations

Bacterial count of milker inflations	Appearance of milker inflations			
	Clean		Not clean	
	No.	%	No.	%
<10,000	242	51.0	34	24
10,000—100,000	97	20.4	25	18
100,000—1,000,000	88	18.3	33	23
>1,000,000	49	10.3	50	35

milking machines examined over the entire course of the study was made to determine to what extent cleanliness by physical examination was verified by the bacterial counts obtained. This included 617 examinations. Of this number, 475 were noted as being clean and 142 as not clean. The bacterial counts of both groups are shown in table 4. In accordance with the data presented, it would appear that milking machines can be judged for bacteriological cleanliness by physical examination with only a fair degree of success, for in 51 per cent of the milkers rated clean, the bacterial counts were less than 10,000, which probably could be considered a "fair" count for inflations stored dry. However, 29 per cent of the milkers that appeared clean were highly contaminated.

There was less relationship between appearance of milker inflations in the "not clean" group. Here 24 per cent had counts of less than 10,000. These figures likely were not representative of average conditions, since the number of samples was relatively small and among them were inflations that frequently were found to retain an oily wax-like deposit as the result of storage in cationic germicide and the inflations of producer 5L

that were doused with warm chlorine solution without first rinsing off adhering milk with clear water.

Producer reaction. Some objections to cationic solutions were expressed by producers. One expressed dislike for cationic detergents because they made the rubber feel "dead" and because they caused the rubber to become coated with a slippery film. Occasionally this would cause the milk pail gaskets to be sucked into the milker pails. It was discovered that this condition occurred mainly when the milk films were not completely washed off the rubber before placing the parts in the solution jar. Lye solution was preferred by this operator because it served better as a detergent.

TABLE 5

The relationship between sanitization treatment and bacterial condition of a single set of milking machine inflations (producer 17L)

Date	Bacterial count/ml.			
	Inflations		Milk	
	Total	Thermoturic	Total	Thermoturic
2-5 ^a	3,000	1,000	10,000	2,400
2-19 ^a	10,000	500	16,000	500
3-27 ^b	310,000	5,000	30,000	400
	510,000 ^c	6,000		
4-3 ^b	52,000	5,000	7,000	300
	200,000 ^c	3,000		
4-10 ^b	1,000,000	2,000	88,000	4,000
	1,300,000 ^c	2,500		
4-16 ^b	10,000,000	2,500	930,000	600,000
	75,000,000 ^c	2,500		
4-24 ^b	30,000,000	25,000	6,000,000	990,000
	21,000,000 ^c	50,000		
5-1 ^a	3,500 ^d	4,500	22,000	400
	10,000	4,000		

^a Hot water used.

^b Cold water used.

^c Rinsed with 200 p.p.m. cationic solution after washing.

^d Stored on solution rack with 200 p.p.m. cationic solution.

Some objection also was expressed with respect to cationic solution forming an oily and somewhat wax-like film when used for sanitizing milker pails. This condition also was found inside inflations. At no time during the course of this study was milkstone a problem. Only during the initial portion of the study was it noticed. The anionic detergent combined with near-neutral polyphosphate was effective in its removal as well as in its prevention. A soft gel-like slime occasionally was found when examining inflations for cleanliness by spatula scraping. This usually was found when chlorine solutions were used for sanitizing without properly removing all milk film by rinsing in water.

Most difficulty with bacterial contamination occurred where washing with detergent and cold or only moderately warm water was used, after which the inflations were stored dry. An illustration of the result of such washing was well demonstrated by producer 17L, who had been producing milk for several months with a good record of low bacterial counts. His hot water heater was sent away for repair for several weeks during the course of the study. Results as shown in table 5 reveal a progressive degree of contamination that first was made evident by increase in the rinse count of inflations. Milk samples remained normal during the first 2 weeks of cold water washing and then greatly increased in total and thermoduric counts. Immediate reduction in counts followed the return to hot water rinsing after washing. Similar patterns were discernible with other producers that washed their machines but did not follow up with effective germicidal treatment. This would support a concept that inflations are not a formidable source of bacteria until contamination has penetrated the rubber pores.

DISCUSSION

The results of storing rubber milker inflations under practical farm conditions indicate that lye and cationic germicide solution storage provide an assurance of better sanitation than does dry storage. Germicidal properties of 0.5 per cent lye and 200 p.p.m. cationic germicidal solutions appear to be of practically equal value where fresh solutions of lye are applied, such as is made possible with solution rack storage. When jar or bath storage was used, cationic solutions provided better sanitizing than did lye. This would indicate that the cationic solutions are more durable as germicides than lye and that when lye jar storage is used for rubber parts, fresh solutions should be prepared more frequently than once each week, as was used in the study.

The cationic solution applied as a rinse following washing was not satisfactory as a means of sanitizing the rubber parts. This was indicated by high bacterial counts in the inflations as well as in milk produced with the inflations. Apparently more intimate exposure to the cationic germicide was required than was possible by drawing 1 gallon of solution through the cups and tubing.

The authors previously reported (3) that rubber inflations were capable of absorbing storage solutions to an appreciable degree. Butterfat and unquestionably bacterial contamination likewise have been found to be absorbed by rubber. With these absorbing qualities definitely shown, it would seem logical to conclude that the problem of sanitizing rubber is not just a matter of treating the surface but of penetrating the pores either by prolonged contact with penetrable germicidal solution or with heat. Hence, the lack of effective germicidal treatment by rinsing and

retaining only a surface film could be expected. It likewise would follow that a combined washing-sanitizing compound, such as frequently has been sought, would not prove effective unless followed by solution storage.

The observations indicate that rinse counts do not represent the entire bacterial contamination but only a portion of that present on the surface of the rubber. Low rinse counts therefore should not be accepted with complete assurance that inflations are sanitized satisfactorily unless the milk produced with them yields low total and thermoduric counts.

In this study, milker pails were not examined for bacterial contamination but only for appearance of cleanliness. General observation led to the belief that the greatest contamination came from milker inflations and tubing.

CONCLUSIONS

Farm application of milker inflation storage employing 0.5 per cent lye solution, 200 p.p.m. cationic germicide and dry storage was observed through means of farm inspection, sterile rinse counts of inflations and bacterial counts of milk. Comparison of storage treatment was made using two different procedures on each of two designated milkers on each farm.

The lye and cationic solutions appeared to have equal germicidal value as measured by total rinse counts when solution rack storage was used. The cationic germicide solution caused greater reduction in thermoduric count of rinse water samples than did lye. Lye solution had less germicidal effectiveness than the cationic germicide when immersion storage was used.

Dry storage was least satisfactory in maintaining uniformly low counts of inflations, and high thermoduric counts were associated with cold water washing followed by dry storage. Dry storage after washing and "sanitizing" with 1 gallon of 200 p.p.m. cationic solution was not satisfactory. Some objection to physical properties of the cationic germicide was registered.

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SWEET POTATO MEAL VERSUS GROUND CORN IN THE RATION OF DAIRY COWS¹

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Raw sweet potatoes have been used as a feed for domestic animals for some time. However, it has been only during the past decade that sweet potato meal has gained recognition as a possible substitute for corn in the ration of livestock. Several southern investigators (6, 7, 8, 9, 10, 11, 12, 13) have found dried sweet potatoes to be worth approximately 90 to 95 per cent as much as corn for fattening beef cattle. Hogs and mules preferred rations in which the sweet potato meal did not replace more than 50 per cent of the corn in the ration. Massey (14) at the Georgia Experiment Station conducted a series of experiments in which he studied the comparative value of sweet potato meal and ground corn as a feed for ewes. The 300 ewes that were fed these rations during a period of 3 years favored the sweet potato meal from a palatability standpoint and gained as much during the gestation period when fed the sweet potato meal ration as when fed the corn meal ration. The ewes that received the sweet potato meal ration produced more milk after the lambs were born and thereby gave the lambs a faster growing start.

Louisiana workers (15) report that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal and are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck, for milk production. Good quality dehydrated sweet potatoes contained from 76 to 81 per cent T.D.N. and a poor quality product contained 71 per cent T.D.N. on the dry basis. Vitamin A analyses of the butterfat from milk produced by cows fed sweet potatoes were 19 per cent higher than those of butterfat produced by cows fed ground corn. The basal feeds were common lespedeza hay, algee clover hay, or kobe lespedeza hay and cottonseed meal. Briggs *et al.* (2) of the Oklahoma Station recently reported that on the dry matter basis the average T.D.N. value of dried sweet potatoes for steers was 86.05 as compared to 85.80 for the corn. The basal feeds were alfalfa hay or prairie hay and cottonseed meal. Copeland (5) of Texas reported that one could expect 3.08 per cent more milk as a result of feeding corn than when feeding sweet potato meal. It also was noted that butter produced from cows on the sweet potato meal contained 37.98 I.U. of vitamin A per g. as compared with 31.11 I.U. from butter produced by cows fed yellow corn.

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Massey (14), in a study with 48 cows, reports that the cows which received the sweet potato meal ration produced 9.3 per cent more milk than did those fed the corn ration.

EXPERIMENTAL PROCEDURE

In the spring of 1946, eight Jersey cows of the University of Georgia dairy herd were paired into two groups (Groups *A* and *B*) and fed comparative rations of sweet potato meal and ground snapped white corn. The constituents of the concentrate mixtures used in the experiment are shown in table 1. The mixtures were the same except that an equal amount of sweet potato meal was substituted for the corn in mixture 2 and equal parts of corn and sweet potato meal were used in mixture 3. The cows also were fed mixed lespedeza and grass hay *ad libitum*. The ani-

TABLE 1
Constituents of grain mixtures used in experiment

Constituents	Mixture 1	Mixture 2	Mixture 3
	(lb.)	(lb.)	(lb.)
Ground snapped white corn	200		100
Sweet potato meal		200	100
Oats	100	100	100
Wheat bran	100	100	100
Cottonseed meal	135	135	135
Bonemeal	9	9	9
Salt	4.5	4.5	4.5

mals were quartered in a dry lot except when being milked by machine twice daily.

The double reversal method of experimentation (1, 4, 18) was employed in this work. Both Groups *A* and *B* were fed mixture 3, which contained equal parts of corn and sweet potato meal, during a preliminary period of 14 days. Then Groups *A* and *B* were fed mixtures 1 and 2, respectively, during the first experimental period. The groups were changed alternately from one ration to the other during the second, third, and fourth experimental periods, each of which extended for 28 days.

When the experiment was about one-third over, cows no. 7 and 8 had to be withdrawn from the experiment due to the incidence of mastitis. In view of the fact that these two cows were paired together before the experiment started, their removal affected the experiment only insofar as the number of cows in each group was reduced by one.

Analysis of variance (4) was used in the statistical treatment of the liveweight and milk and butterfat production data according to two different methods. Method 1 tripled the differences between the response of the cows on the two feeds during the second and third experimental periods ($-a + 3b - 3c + d$) and gave equal weight to the differences during the first and

fourth periods. Method 2 (3) consisted of giving equal weight ($a-b + c-d$) to the differences incurred during each experimental period.

RESULTS

Chemical analysis of feeds. Sufficient quantities of both rations were mixed bimonthly so as to keep a fresh supply of feed on hand at all times. A representative sample from each batch of feed was analyzed for dry matter, ether extract, crude protein, nitrogen-free extract, crude fiber, ash and moisture. The data shown in table 2 reveal that the corn ration contained 0.70 per cent more moisture, 1.6 per cent more fiber, 0.50 per cent more protein, 0.30 per cent more fat, 1.0 per cent less ash and 2.20 per cent less nitrogen-free extract than did the sweet potato meal ration. Very little difference in the chemical composition of the two mixtures was apparent.

The work of Briggs *et al.* (2) and Rusoff *et al.* (15) indicates that the

TABLE 2
*Average chemical analyses of experimental rations**

Mixture	Constituents					
	Moisture	Fiber	Protein	Ash	Fat	Nitrogen-free extract
1	9.8	8.8	16.9	5.2	4.3	55.0
2	9.1	7.2	16.4	6.2	4.0	57.2

* Chemical analyses were made by personnel of the State Chemists Department.

apparent digestion of coefficients of the protein, fat and fiber of dehydrated sweet potato meal may be rather low. The Louisiana workers (15) point out that since the N.F.E. made up over 84 per cent of the dry matter in the dehydrated sweet potato, the apparent lack of digestibility of the other constituents had but little effect on the T.D.N. content and that the low content of protein precludes this product from being an important source of this nutrient. It would appear from these reports (2, 15) that the sweet potato meal ration (mixture 2) fed in this experiment may have been a little lower in digestible protein than the corn ration (mixture 1).

Palatability. All of the cows relished the sweet potato meal (Porto Rico variety) ration from the start of the experiment; when the cows were changed from one ration to the other, they did not eat the corn ration as readily as they did the sweet potato meal ration. This is in agreement with the results obtained by Massey (14) of the Georgia Experiment Station and Seath (16) and Seath *et al.* (17). The Porto Rico variety of sweet potato was fed in these studies. The Louisiana workers (17) observed in another study that, when the high-starch sweet potato variety, L-45, was compared with ground yellow corn meal in the ration,

from 1 to 4 days were required for all of the cows to become accustomed to the change in the rations. Then they ate the ration containing sweet potato meal as readily as they did the one containing corn meal. In regard to the varying degrees of palatability of sweet potato meal as reported, one should remember that this product is a relatively new livestock feed, the quality of which has not yet been standardized. The proportion of sweet potato meal to other constituents and the number of various ingredients used in the ration probably would have a definite relationship to the palatability of the ration containing the sweet potato meal. From the standpoint of color and palatability, the product used in this experiment was excellent.

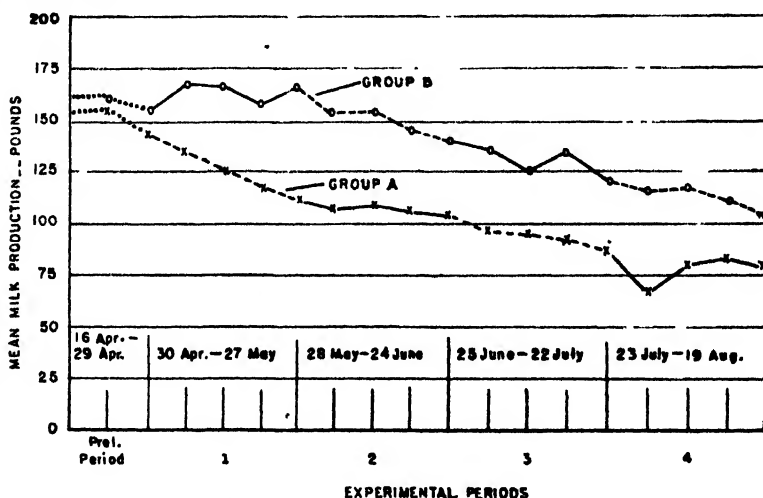


FIG. 1. Curves showing mean milk yield of cows on experiment. (Group A = X; Group B = O. Mixture 1 = -----; Mixture 2 = —————; Mixture 3 =)

The cows fed the sweet potato meal appeared to have a sleeker hair coat than did those fed the corn ration. There was no indication of any unusual looseness in the feces of the animals as the result of feeding the sweet potato meal; the feces of these cows were not as loose as those from the regular herd cows which were on green pasture. Massey (14) likewise found no digestive disturbances among the cows fed sweet potato meal, although it seemed to have a more laxative effect than did the corn meal.

Liveweight. The liveweight average per cow when fed the corn ration was 798 lb. as compared to 801 lb. when fed the sweet potato meal ration. Statistical treatment of the data according to the methods described previously gave *F*-tests which indicate that there was no significant difference between the effect of the two feeds on the liveweights of the cows.

Milk and butterfat yields. A comparative study of the effect of corn and sweet potato meal on milk and butterfat production was made during each of the experimental periods. The milk production data (Fig. 1) reveal that although the level of production of Group B was higher than that of Group A, the trend of the lactation curves of the two groups of cows was very much the same throughout the experiment. The level of production of the two groups was about the same at the start of the study. The sharp drop in the curve of Group A during the period July 23 through July 29, inclusive, was attributed to a case of foot rot, which one of the cows had during that period. Group A was being fed sweet potato meal ration during this period.

From the standpoint of total production, when the cows were fed the corn ration (mixture 1) they produced 5,752.1 lb. of milk as compared to 5,741.2 lb. when fed the sweet potato meal ration (mixture 2). The butterfat yields of the cows when fed each of the experimental rations were calculated from a bimonthly butterfat test and the actual milk production. When fed the corn ration, the cows produced a total of 267.58 lb. of butterfat as compared to 268.72 lb. when fed the sweet potato meal ration. The *F*-tests indicate that there was no significant difference between the effects of the two feeds on the amount of milk and butterfat produced.

SUMMARY

A comparative study of the effect of ground snapped white corn and sweet potato meal on the liveweight and milk and butterfat production of dairy cows was conducted during the spring and summer of 1946. Analysis of variance revealed no significant differences in the milk and butterfat production or in the liveweights of cows when the sweet potato meal or corn constituted 36 per cent of the concentrate mixture. The sweet potato meal was as palatable as the ground corn when each of these concentrates constituted 36 per cent of the concentrate mixture. No excessive or objectionable laxative effect upon the digestive system of the cows was noted when they were fed the sweet potato meal mixture. The cows fed the sweet potato meal had a sleeker, brighter hair coat than did those fed the corn ration.

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CORRELATION BETWEEN THE LACTOSE CONTENT OF MILK AND THE CEREBROSIDE AND CHOLINE CONTENT OF BRAIN

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Although the composition of cerebrosides is well known, their function is not understood. They are found in almost all tissues but are present in largest amounts in the brain. As the cerebrosides are relatively stable under various conditions (14, 15), they are believed to function as basic structural elements. Although the cerebroside composition of different brains has been studied (9, 12, 14, 15), a systematic study of their relation to other lipids has not yet been made.

The cerebrosides have a galactose residue; sphingomyelins have a choline phosphoric acid residue. This led to the belief (1) that the two radicals might be interchangeable, that galactose might act as a choline sparer in the organism. If true, this would throw some light on the functions of lactose in milk, and also on the functions of cerebrosides and sphingomyelins. If there is such sparing of choline by galactose, it may be of significance in lipotropy and related phenomena. This problem was studied by feeding different levels of lactose. However, large amounts of lactose exert an unfavorable effect, apparently by competition with the glucose at the tissue centers where glucose is metabolized. A report (13) appeared on the effect of lactose feeding on the cerebroside content of the rat brain, but not on the change in choline distribution. Therefore, the author has attempted to find out if there is a correlation between the lactose percentage in milk and the cerebroside content of the brain in several species of mammals.

METHODS

The brains of the various freshly killed animals were weighed, dried in a vacuum oven for 48 hours, pulverized, weighed, and extracted with absolute methanol in a Soxhlet apparatus for 36 hours. The choline in the extract was determined by the method of Glick (6) and the cerebrosides by the method of Brückner (2, 3). Figures 1 and 2 and table 1 show the correlation between choline and galactose in the brain and the lactose in the milk. The effects of age, gestation, and cortical differentiation on the cerebrosides and choline contents of the several brains are presented in tables 2 to 4.

RESULTS AND DISCUSSION

No definite major function has yet been ascribed to galactose, although many minor functions have been attributed to it (1). Biochemically,

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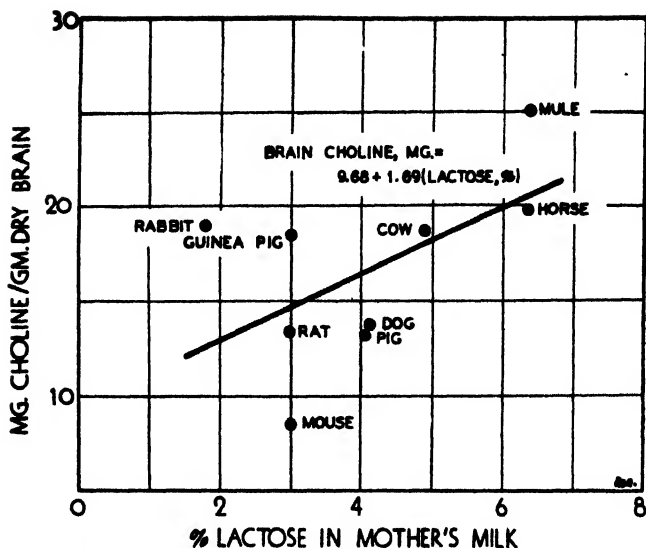


Fig. 1. Correlation of brain galactose with lactose percentage in milk of different species. (Each data point represents one animal.)

galactose is more resistant to oxidation in the body than is glucose. This greater stability of lactose led to the belief that galactoses form a hydrophilic group attached to the sphingosine base. As glucose may form a

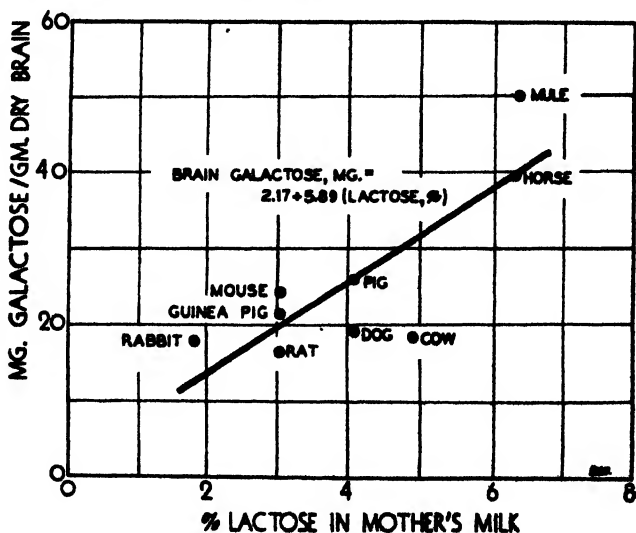


Fig. 2. Correlation of brain choline with lactose percentage in milk of different species.

similar hydrophilic group, gluco-cerebrosides would be expected to exist in the body, and, indeed, gluco-cerebrosides have been isolated from the spleens of patients suffering from Gaucher's disease (5, 7, 11) though the normal galacto-cerebrosides also were present (10). Since then gluco-cerebrosides also have been found in the normal cattle spleen (18) but never in the brain. It is likely that gluco-cerebrosides normally are present side by side with galacto-cerebrosides but, since glucose is the only oxidative substrate for the brain, it is quickly oxidized, and, therefore, not detectable in the brain. Galactose, on the other hand, being resistant to oxidation, forms stable galactosides of brain cerebrosides. This assumption is substantiated by the presence of both gluco- and galacto-cerebrosides in the spleen (8) but only galacto-cerebroside in the brain.

TABLE 1
Choline and galactose content of cerebrosides

Species	No. estimations	Choline (% of dry matter)	Galactose (% of dry matter)
Rat	24	1.346	1.667
Mouse	12	0.846	2.456
Rabbit	18	1.878	1.797
Pig	10	1.306	2.621
Guinea pig	10	1.831	2.133
Dog	6	1.368	1.915
Cow	12	1.826	2.239
Horse	6	1.979	3.995
Mule	10	2.507	5.053

It is true that galactose is synthesized by all animals, but in the mammalian series preformed galactose, supplied by lactose, is likely to determine the galactoside content in the phylogenetic scale of evolution. Feeding of lactose in nontoxic amounts increases galactoside formation in the rat brain (13).

If the galactose can replace the choline phosphoric acid of sphingomyelins, an inverse relationship may be expected between galactose and sphingomyelin contents of brain. Data on rat brain lipids (14, 15) show that as cerebrosides increase with age, sphingomyelins decrease. However, this is true only when these analyses are expressed as percentages of total brain lipids; both cerebrosides and sphingomyelins show an increase in absolute amounts with increasing age. Figure 2 shows that when the choline contents of the brains of different mammals are compared with the lactose contents of milk, a positive correlation is obtained, although a negative correlation was expected. Even if sphingomyelins decrease with increase of galactosides, there may be a simultaneous increase in other choline-containing lipids. As sphingomyelins were not estimated, this question cannot be settled. However, it should be noted, that the correlation between lactose and choline is not very high, and, if a concomitant increase

in lecithins or choline fractions other than sphingomyelins occurs, a negative correlation may be found between lactose and sphingomyelins. It would be interesting to estimate the galactose and sphingomyelin content of the brains of experimental animals fed graded amounts of galactose and choline.

TABLE 2
Influence of age on choline and galactose content of brains

Species	Age	Choline (% of dry matter)	Galactose (% of dry matter)
Rat	8 days	2.244	1.385
	14 days	1.718	1.304
	Adult	1.346	1.667
Mouse	20 days	1.630	1.180
	Adult	0.846	2.456
Rabbit	22 days foeti	1.477	1.599
	Adult	1.878	1.797

TABLE 3
Influence of gestation on choline and galactose content of brain

Species	Choline (% of dry matter)		Galactose (% of dry matter)	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
Rat	1.346	1.493	1.667	2.568
Rabbit	1.878	1.274	1.797	2.684

TABLE 4
Choline and galactose content of cortex and medulla as compared to that of the composite sample of the brain

Species	Choline (% of dry matter)			Galactose (% of dry matter)		
	Cortex	Medulla	Composite	Cortex	Medulla	Composite
Cow	1.755	1.833	1.826	2.185	2.243	2.239
Dog	1.337	1.378	1.368	2.053	1.902	1.915

Table 2 shows that there is an increase in the cerebroside content of the brain with age, confirming earlier results (14, 15). Table 3 shows an increase in brain cerebroside with increasing period of gestation, as might be expected, since lactose formation begins in the mother during the gestation period. Table 4 shows no difference in cerebroside content of different species associated with difference in the degrees of cortical differentiation or with different proportions of cortical and medullary tissues.

SUMMARY

1. Nine species of mammals have been examined for the cerebroside and choline contents of their brains. Brain cerebroside shows a high positive correlation with the lactose percentage of respective milks. There

is a slight positive correlation between the choline content of brains and the lactose content of the mothers' milk.

It is suggested that sphingosine base can reversibly take up galactose or choline phosphoric acid to form cerebrosides or sphingomyelins and, thus, cerebrosides may function as choline spacers.

2. There is an increase of cerebrosides with age and gestation. There is no significant difference in the cerebroside distribution between cortex and medulla of the brains of the species studied.

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ESTIMATING THE AMOUNT OF FEED DERIVED FROM PASTURE BY COWS IN THE CONNECTICUT DAIRY HERD IMPROVEMENT ASSOCIATION

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In many problems of farm management, estimates of the contribution of pasture to total annual feed consumption of dairy cows are desirable. The most common methods for determining pasture yields may be classified broadly under three headings: (a) Yields per acre of pasture based on agronomic data, a procedure which involves clipping, drying, and chemical analysis of the pasture grasses. (b) By quantitative grazing, which measures the weight changes of livestock and involves estimating the yield of pasture from the recorded weight changes. (c) On the basis of milk production records, when total feed requirements are estimated, barn feeds are known, and pasture appears as a residual. The purpose of this paper is to enlarge on the latter method.

EXPERIMENTAL PROCEDURE

A measure of energetic efficiency of milk production which describes the input-output relationship of milk production was selected. The measure was applied to production and feed consumption records by month of lactation for cows freshening in the winter months when barn feed equaled total feed input. The computed efficiencies of milk production for the various months of lactation were applied to the milk production records for cows freshening in the various months and estimates were made of the total nutrients required by month of lactation. After the total nutrients required were computed by month of freshening and month of lactation, the contribution of pasture was determined by deducting the known barn feeds from the estimated total requirements.

A measure of energetic efficiency of milk production. To obtain an estimate of a cow's consumption of total digestible nutrients (T.D.N.), certain measures which describe the cow's ability to convert feed into milk are used. These measures of energetic efficiency of milk production are based on records of milk output, feed intake, weight of cows, and so on.

Various types of dairy-merit indices have been developed. Davis *et al.* (3) proposed a dairy-merit index relating fat-corrected milk (F.C.M.) and weight (W). Brody and Nisbet (2) and Kleiber (7) relate

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milk production to the three-fourths power of body weight. Other indices relating the amount of feed consumption and the amount of milk production, however, are much more applicable to this analysis. Brody (1) has developed a relationship which defines dairy merit as the ratio of milk-energy production to T.D.N. energy consumption. Based on the ratios of 1 lb. of milk to equal 340 calories and 1 lb. of T.D.N. to equal 1,814 calories, the relationship is:

$$\text{Dairy-merit ratio} = \frac{340 \times \text{lb. 4\% F.C.M. produced}}{1,814 \times \text{lb. of T.D.N. consumed}}$$

Brody used this for comparing milk producing capabilities of various cows within breeds and between breeds and also to compare different mammals. He found that dairy merit varied between different mammals and that the larger breeds of dairy cows had a lower dairy merit than the smaller breeds.

Dairy merit by month of lactation. Brody's analysis and use of the concept of dairy merit was entirely on an annual basis. In this study his measure is adapted to monthly input-output data in order to develop dairy-merit indices which lead to a determination of the total T.D.N. consumption and the feed derived from pasture. With the established measures of dairy merit by month of lactation, records of milk production may be used to derive monthly feed requirements. Deducting recorded amounts of T.D.N. in barn feed from these required nutrients should give the contribution of pasture.

The records from ten D.H.I.A. herds over a period of 10 years from 1935 through 1944 served as the source for input-output records. A total of 1,200 lactation records from Holstein cows was obtained, with 100 records in each month of freshening. While these records are subject to the usual limitations in applying a single day's performance to estimate an entire month, the records of concentrate feeding were taken on an individual basis and appeared to be reasonably accurate. The records of barn-fed roughages, however, were recorded as the average of several cows or the entire herd. This method of computing roughage consumption would tend, among other things, to obscure any possible differences that may have been associated with the stage of lactation.

The input-output relationships developed from D.H.I.A. data were based only on those months when pasture was unimportant. This was done on an average basis by combining figures for the barn-feeding months from December through March for all months of freshening, and so obtaining average inputs and outputs for each month in the lactation cycle.

RESULTS

The dairy-merit ratios for the different months of lactation, calculated according to Brody's formula, are given in table 1. Since feed inputs

do not decline as rapidly as milk production during the lactation period, dairy merit decreases throughout the period. Disregarding the results for the first partial month, dairy merit averaged about 34 per cent during the second month and 20 per cent during the ninth month, an average decline of 2 per cent per month. The tenth through twelfth months of lactation are subject to more rapidly declining milk production and dairy merit, presumably as a result of advancing gestation.

TABLE 1
Estimates of dairy merit by months during the lactation period

Mo. of lactation	Av. lb. of milk per month ^a	Av. lb. of T.D.N. per month ^a	% dairy merit
1	448	195	43.3 ^b
2	1160	635	34.2
3	1048	626	31.3
4	958	614	29.2
5	870	594	27.4
6	799	576	26.0
7	736	558	24.7
8	650	538	22.6
9	557	520	20.1
10	393	490	15.0
11	267	475	10.5
12	162	456	6.7

^a Milk production has been converted to 4% F.C.M., while feed inputs have been converted to pounds of T.D.N. on the basis of 0.75 per lb. of grain, 0.50 per lb. of hay, and 0.18 per lb. of ensilage.

^b Based on production and feed for an average of 10.8 days during the month. Since feed inputs were allocated in the ratio of 10.8 to 30, the amount fed after freshening probably is underestimated and the calculated lactational efficiency correspondingly high.

Estimating required nutrients, and the contribution of pasture. The dairy-merit ratio defined by Brody may be converted to give directly the amount of feed obtained from pasture. The formula is as follows.

$$\text{Pounds of T.D.N. from pasture} = \frac{340 \times \text{F.C.M.}}{1,814 \times \text{D.M.}} - \text{Pounds of T.D.N. from barn feed}$$

where D.M. represents the previously calculated dairy-merit ratios.

A first attempt at an estimate of pasture will consider the feed equivalent obtained from pasture for cows freshening in the month of January. Table 2 summarizes the computations. These estimates indicate that such cows obtained about 6 per cent of their April feed requirements from pasture, 50 per cent of their May requirements, 74 per cent of their June requirements, and so on. On an annual basis, pasture accounted for 37 per cent of the total feed requirements.

Monthly feed requirements from cows calving in each month of freshening were computed in a manner similar to that for cows freshening in January. Having computed the feed requirements and the breakdown of source of feed, whether from pasture or from barn feeds, it is possible

to combine feed records by month of lactation and by month of freshening. The monthly composite feed records for a herd organization with equal numbers of cows freshening in each of the calendar months may be obtained in this manner. Table 3 summarizes the amounts of recorded nutrients from grain, hay and ensilage, and the total required T.D.N. based on milk production. The amount of pasture was found by deducting the total recorded T.D.N. per day from the total required T.D.N. per day. When the cows are not getting pasture, this figure would be expected to

TABLE 2

Estimates of monthly feed requirements and nutrients obtained from pasture for cows freshening in January

Month	Fat-corrected milk per month	Barn-fed T.D.N. per month	Estimated T.D.N. ^a		
			From all sources	From pasture	
	(lb.)	(lb.)	(lb.)	(lb.)	(%)
Jan. ^b . . .	457	556	543	- 13 ^c	- 2.4 ^d
Feb.	1058	597	580	- 17	- 2.9
March . . .	1079	633	646	+ 13	+ 2.0
April . . .	951	571	610	+ 39	+ 6.4
May	980	332	670	+ 338	+ 50.4
June . . .	906	172	653	+ 481	+ 73.7
July	825	179	626	+ 447	+ 71.4
Aug.	756	181	627	+ 446	+ 71.1
Sept. . . .	654	207	610	+ 403	+ 66.1
Oct.	536	276	670	+ 394	+ 58.8
Nov. . . .	306	435	546	+ 111	+ 20.3
Dec. . . .	186	478	520	+ 42	+ 8.1
Total . . .	8694	4617	7301	2684	36.8

^a Using estimated dairy merit from table 1.

^b Since cows in the sample freshened throughout the entire month, the data applied to an average period of only 10.8 milking days.

^c Difference between T.D.N. from all sources and barn-fed T.D.N.

^d Per cent pasture is of total feed requirements.

be zero and any plus or minus amounts would be due to inaccuracy of the method. The fact that these differences are quite small, however, suggests that the errors in the use of this method are not large.

On an annual basis (table 3), considering a herd with equal numbers of cows freshening in each of the calendar months, pasture accounts for 36 per cent of the total T.D.N., grain contributes 27 per cent, hay 21 per cent and ensilage 16 per cent of the total nutrients. The contribution of pasture to total feed requirements of cows freshening in the various months varies from a high of 38 per cent for cows freshening in March to a low of 33 per cent for cows freshening in August. For brevity, tables similar to table 2 are not presented for cows freshening in the months from February through December.

TABLE 3
*Estimates of nutrients obtained from pasture in different months on 10 D.H.I.A. farms
in Connecticut, based on 10-year records of production of milk and
consumption of grain, hay, and ensilage*

Item	Calendar months												Total for year (lb.)	% of total T.D.N.
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
	(ar. lb. per day)													
<i>Milk produced</i>	22.1	22.0	22.5	23.0	25.6	25.9	24.3	24.2	24.1	23.5	21.7	22.0		
<i>4% F.C.M.</i>	18.2	17.9	18.4	18.9	20.9	21.8	20.1	19.8	20.1	19.7	18.0	18.4	6966.0	
<i>Total required T.D.N. Based on milk production</i>														
<i>T.D.N. furnished & recorded</i>														
Grain ..	5.5	5.9	5.8	5.9	5.1	4.6	4.7	4.7	4.9	5.1	5.4	5.4	1890.0	27.1
Hay ..	7.4	7.6	7.6	7.0	2.6	0.2	0.2	0.2	0.5	1.6	6.4	7.5	1464.0	21.0
Ensilage ..	5.2	5.1	4.7	4.2	1.6	0.3	0.6	0.9	2.1	3.4	4.7	5.3	1143.0	16.4
<i>Total</i>	18.1	18.6	18.1	17.1	9.3	5.1	5.5	5.8	7.5	10.1	16.5	18.2		
<i>T.D.N. furnished by pasture</i>														
Difference between required and recorded T.D.N.	0.1	-0.7	0.3	1.8	11.6	16.7	14.6	14.0	12.6	9.6	1.5	0.2	2469.0	35.5
% of required T.D.N. fur- nished by pasture	(0.5)	(-3.9)	(1.6)	9.5	55.5	76.6	72.6	70.7	62.7	48.7	8.3	(1.1)		

DISCUSSION

There are several limitations to the measure used in this analysis. As stated by Brody, dairy merit varies from cow to cow and is affected by such factors as changes in body weight and size. Since no weight data were available for the D.H.I.A. records used in this analysis, changes in body weight due to overfeeding or loss of flesh as well as to natural growth may have influenced the dairy-merit indices. Dairy merit is affected by age, which includes a joint relationship of increased body size and advanced maturity as related to increased milk production. This relationship is particularly important when working with lactation curves, since the slope of the production curve varies for cows of different ages; therefore, results are affected by the age of the cows in the sample. Dairy merit is a function of feeding levels and not a constant; the curvilinear total input-output curve based on smoothed data as given by Jensen *et al.* (6) would correspond to dairy-merit ratios increasing from 23.0 per cent at 6,000 lb. of 4 per cent F.C.M. output to 25.4 per cent at 8,000 lb. of 4 per cent F.C.M., and then decreasing to 23.6 per cent at 10,000 lb. of 4 per cent F.C.M. Dairy merit varies with environment, especially temperature. Regan and Richardson (9) found that higher temperatures had an adverse effect upon milk production. Some degree of error in the estimation of pasture could be explained on the basis of temperature, since dairy-merit indices were based on the winter months when temperature, as well as the other environmental conditions, was fairly constant.

While the measure under discussion has these limitations, it may prove particularly useful in two cases. First, as shown above, an estimate of the pasture consumption of a herd can be computed. Second, this measure could be used as a means of estimating pasture yields on a seasonal per acre basis. This would provide another measure for estimating pasture consumption on individual farms as well as an additional check on pasture studies involving lactating dairy cows, in which clipping and grazing methods are used. No significant changes would need to be made in the managerial techniques now used in the grazing methods as employed by Hodgson and Shepherd (5) or in the procedures recommended by the Joint Committee of the American Society of Agronomy, American Dairy Science Association and American Society of Animal Production (8). The results, however, would provide another comparison between the clipping (actual growth) and the grazing (actual consumption) methods. Hodgson *et al.* (4) found some divergence in the two methods which might be verified and explained by the use of a third comparison.

SUMMARY

The residual method was used in order to determine the contribution of pasture to total feed supply for a sample of 1,200 Holstein cows from

10 D.H.I.A. farms in Connecticut. One of Brody's measures of dairy merit was combined with the input-output records of the 1,200 cows. Dairy-merit indices were computed for each month of lactation on the basis of winter feed and production records. Pasture contributions then were determined by month of lactation for cows freshening in the various months. Pasture accounted for 38 per cent of the total feed for a cow freshening in March and 33 per cent of the feed for a cow freshening in August, on an annual basis. These were the highest and the lowest percentages, respectively.

On a yearly herd basis, using an equal number of cows freshening in each month, pasture accounted for 36 per cent of the total T.D.N. intake, grain for 27 per cent, hay for 21 per cent, and ensilage for 16 per cent of the total nutrients.

While there are limitations, this method may be used in computing pasture yield on a seasonal per acre basis. This would provide an additional check on pasture studies in which clipping and grazing methods are used.

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EFFECT OF WATER SPRINKLING WITH AND WITHOUT AIR MOVEMENT ON COOLING DAIRY COWS

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The knowledge that cattle produce some insensible perspiration yet lack true sweat glands (1) has caused a great deal of speculation among livestock men as to just how much effect artificial sprinkling or wetting by rain may have on cooling cows during warm weather. Likewise, there is much difference of opinion as to how much effect air movement, such as wind, may have on the cooling of cows. This applies to cows prior to sprinkling as well as after sprinkling.

In a previous experiment at this Station (4) it was found that milk cows when removed from the sunshine to shade cooled much faster when sprinkled with water than when not sprinkled. Body temperatures for those sprinkled reduced to normal levels in 1 hour, while those not sprinkled averaged approximately 0.5° F. higher. Also, the reduction in respiration rate was almost twice as great for those sprinkled as for those not sprinkled. Unpublished results of other Louisiana Station experiments (3) have shown that natural rain tends to cool milk cows rapidly. Experimental work in India (2) has shown this same tendency, with the body temperatures of water buffaloes, hill cattle, and sheep dropping considerably and those of zebu cattle showing somewhat less reduction due to heavy natural showers. It was found that wetting animals by hosing for 3 minutes was as effective in cooling water buffaloes as was allowing them to wallow for 20 minutes or for 1 hour.

In a later Indian experiment (5), 15 water buffaloes produced significantly more milk when wetting of their bodies was carried on by splashing. The authors concluded that body wetting was essential for water buffaloes during the hot months.

As reported by Kendall (1), the water lost by insensible perspiration by cattle on a maintenance ration was two to three times greater than that passed in the urine. This loss, he reports, may vary 12 or more lb. per day, with decreases accompanying a drop in air temperature. This large moisture loss, while not true sweat, appears to be relatively important, and the variations normally occurring may be associated with air movement as well as with changes in air temperatures. This experiment was designed to determine the importance of these factors plus others associated with air movement and body sprinkling on the cooling of dairy cows.

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EXPERIMENTAL PROCEDURE

Six Jersey cows in milk were utilized in a 2×2 factorial design (6), being divided as equally as possible into two groups of three cows each. Twelve warm clear days between July 11 and August 5, 1947, were selected for conducting this experiment. On each day selected all six cows were tied outside in the sun between 12:00 noon and 2:00 p.m., after which records were made of respiration rates taken from flank movements and of rectal body temperatures. Following this, three of the cows were completely wet by use of a hand sprinkler containing water averaging approximately 85° F. in temperature. Then the cows were put into a special section of the experimental barn. This section was enclosed and rectangular in shape with a 40-inch exhaust fan at one end. Panel-like doors could be opened at the far end when the fan was running to permit circulation of air. The area was divided into three stalls but the sides of the stalls were not solid, thus allowing air circulation. Two cows were tied in

TABLE 1

Factorial design for determining effectiveness of water sprinkling on cooling dairy cows (6 Jersey cows)

Test days	Fan operation	Sprinkling procedure ^a	
		Group A	Group B
1, 5, 9	Without fan	Dry	Wet
2, 6, 10	Without fan	Wet	Dry
4, 7, 11	With fan	Wet	Dry
3, 8, 12	With fan	Dry	Wet

^a 3 cows in each group.

each stall, one at each end, and changes from stall to stall were made systematically from day to day. On half of the days the fan was started as soon as the cows entered the stall and continued running for 1 hour. During the other days the cows remained in the stalls without the fan being in operation. Tests made for wind velocity by use of a Taylor Biram-type anemometer were rather unsatisfactory but showed practically no detectable movement near the floor and from 40 to 240 feet per minute at levels between the cows' bellies and their backs. The division of the cows and treatments is given in table 1.

As shown, each treatment was performed on three different test days. This particular design, as can be seen, has the effects of fan versus no fan confounded with differences caused by variations between test days (6). The effects of treatments as described were determined by noting changes in body temperature and respiration rate after cows had remained 0.5 hour and 1 hour in the special section of the experimental barn.

RESULTS

Records on cows while in sun. After the cows had remained in the

TABLE 2

Average body temperatures and respiration rates of individual cows after standing in sun for 2 hours

Cow no.	Body temperature (°F.)		Respiration rate (times per min.)	
	Range	Av.	Range	Av.
X1	102.1-104.7	103.47	80-130	106.3
4	101.5-103.4	102.46	66-115	89.3
X5	101.3-105.1	103.36	92-138	112.5
12	103.8-107.4	105.91	136-175	153.9
X4	101.2-104.0	102.84	75-132	104.9
X7	101.5-104.6	102.92	68-106	86.8
Av.		103.49		109.0

sunshine for 2 hours, the rectal body temperatures (table 2) ranged from 101.2 to 107.4° F. and averaged 103.49° F. The average temperature prior to the cooling treatments thus was approximately 2.0° F. higher than that which usually is considered normal. Respiration rates varied from 66 to 175 per minute, with an average of 109. This average was more than twice as high as were the respiration rates during cool weather.

The variations shown in these preliminary records prior to cooling appear extremely large. Much of this variation was due to differences

TABLE 3

Comparative average reductions in body temperatures of cows due to wetting and air movement

Test days	Atmospheric records at 2 p.m.		Decrease in body temperature at end of 1 hr.	
	Temperatures	Humidity	Dry group	Sprinkled group
	(° F.)	(%)	(° F.)	(° F.)
Without fan				
1	90	36	0.63	0.83
2	92	45	0.77	1.77
5	94	46	0.53	1.07
6	87	29	0.47	1.07
9	93	50	0.70	1.07
10	92	48	1.10	1.77
Av.	91.3	42.3	0.70	1.26
With fan				
4	92	42	1.47	1.77
3	90	61	1.00	1.43
7	97	47	1.30	1.77
8	94	49	1.33	2.03
11	94	43	1.70	1.90
12	96	44	1.53	2.17
Av.	93.8	47.7	1.39	1.84

between individual cows as well as to the day to day variability found in air temperatures (table 3). Air temperatures ranged from 87 to 97° F. and averaged 91.3° F. on days when the fan was not used and 93.8° F. on the days when the fan was used.

Influence of cooling on body temperatures. Statistically significant differences (6) were found in favor of sprinkling as contrasted to not sprinkling and in favor of air movement caused by fan as compared to no fan (tables 3, 4 and 5). Cows left dry without fan showed an average decrease in body temperature after 0.5 hour of 0.28° F. as contrasted to 0.62° F. with fan alone, 0.83° F. for sprinkling alone, and a decrease of 1.42° F. for cows receiving both sprinkling and fan. At the end of 1 hour for the non-sprinkled and non-fan treatment, the decrease in body temperatures averaged only 0.70° F., and for sprinkling plus fan, 1.84° F.

TABLE 4

Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 0.5 hour

Cow no.	Av. reduction in body temperature after 0.5 hr.				Av. reduction in respiration rate after 0.5 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° F.)	(° F.)	(° F.)	(° F.)	(times per minute)			
X1	0.93	0.53	0.57	1.30	24.0	35.7	35.3	41.3
4	0.33	0.57	0.73	0.83	25.0	32.0	22.0	52.0
X5	0.33	0.97	0.90	1.23	25.7	30.0	37.7	40.7
12	-0.10	1.70	0.53	2.47	-3.0	64.3	18.0	43.0
X4	0.10	0.57	0.37	1.50	16.7	31.0	26.7	48.0
X7	0.40	0.63	0.63	1.20	10.0	30.7	23.0	29.0
Av.	0.28	0.83	0.62	1.42	16.4	37.3	27.1	42.3

The trend appears to be consistent whether considered for individual test days (table 3) or for individual cows (tables 4 and 5), except in the case of the cows sprinkled without fan; those cows had cooled more at the end of 0.5 hour than had the dry cows with the benefit of fan. This was reversed, however, at the end of 1 hour, when the fan-treated cows were the cooler, thus showing the slower cooling effect produced by the fan alone.

Influence of cooling on respiration rates. For cows receiving the sprinkling treatment in this experiment, respiration rates per minute decreased more on an average at the end of 0.5 hour of cooling (table 4) than at the end of 1 hour (table 5). The cows left dry either with or without fan failed to follow this trend, i.e., those without fan decreased an average of 16.4 respirations per minute at the end of 0.5 hour and 20.8 at the end of 1 hour. With fan, the decrease was 27.1 at the end of 0.5 hour and 37.1 at the end of 1 hour. In contrast, those sprinkled decreased

37.3 at the end of 0.5 hour and 31.8 at the end of 1 hour; those with sprinkling and fan reduced 42.3 times per minute in 0.5 hour and 35.3 at the end of 1 hour.

Cow no. 12 (table 4), on an average, failed to decrease either in respiration rate or body temperature at the end of 0.5 hour when left dry and without fan. At the end of 1 hour a slight average reduction in respiration of 3 times per minute was shown, while body temperature also showed an average decrease. This particular cow suffered much from exposure to sunshine and had high body temperatures and respiration rates prior to

TABLE 5

Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 1 hour

Cow no.	Av. reduction in body temperature after 1 hr.				Av. reduction in respiration rate after 1 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° F.)	(° F.)	(° F.)	(° F.)	(times per minute)			
X1	1.13	1.20	1.53	1.97	34.3	35.0	44.0	40.3
4	0.43	0.83	1.00	1.27	21.7	31.3	38.7	41.3
X5	0.77	0.93	1.80	1.87	28.0	25.7	47.3	34.0
12	0.77	2.90	1.27	2.70	3.0	64.0	36.7	34.3
X4	0.40	0.80	1.30	1.70	23.3	15.3	29.0	39.7
X7	0.70	0.90	1.43	1.57	14.7	19.7	26.7	22.3
Av.	0.70	1.26	1.39	1.84	20.8	31.8	37.1	35.3

cooling. The fact that she had calved only shortly prior to the start of the experiment partially explains her unusual reactions.

DISCUSSION

Results from this experiment give information in addition to that already published (4) concerning the cooling of cows in Louisiana. Of particular interest is the added information that the circulation of air by a fan greatly increases the cooling of both the non-sprinkled and sprinkled cows. For the non-sprinkled cows fanning increased the cooling rate by convection because the ambient air temperature was below that of the body. Perhaps evaporation of insensible perspiration also is a factor, but this is a problem which remains to be investigated. There is need for partitioning the heat loss between convection, vaporization, and radiation, all of which are affected by the vapor pressure of the air and skin and by the air movement.

These trials showed that the least cooling of cows took place without any sprinkling or air circulation and the best results were secured when cows were first sprinkled and then subjected to air circulation. The use of sprinkling alone or fan alone produced essentially equal results, with

sprinkling producing the greater drop in both body temperature and respiration rate at the end of 0.5 hour (table 4) and the fan being the more effective by the end of 1 hour (table 5). In these comparisons it is of interest that sprinkling alone produced a lower respiration rate at the end of 0.5 hour than was present after 1 hour. This was not true for body temperature, which is much slower to respond to cooling treatments. The fact that the cows were sprinkled just once and with water at approximately 85° F. probably helps explain why the maximum reduction in respiration was secured at the end of the 0.5-hour period.

SUMMARY AND CONCLUSIONS

When cows were removed from sunshine, sprinkled with water and then subjected to a gentle breeze produced by a fan, they showed rapid changes toward normal body temperature and respiration rate. Shade alone showed a small change in that direction, while the fan alone and sprinkling without a fan were intermediate in their effects.

The results of this experiment give valuable information on how rain and wind as produced by nature tend to cool milking cows during summer months. The results also suggest the need for further experimental work on how mechanical sprayers and fans may be utilized economically during summer when nature is not producing wind or rain.

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MEASUREMENT OF FLUORESCENT MATERIALS IN MILK AND MILK PRODUCTS¹

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A number of workers in recent years have shown that various dehydrated food products and extracts thereof emit blue fluorescence when subjected to ultraviolet radiation. The intensity of fluorescence increases during storage of the products, and, in some cases, is correlated with loss of palatability, as Fryd and Hanson (8) found with dried eggs. Others, notably at the National Research Council of Canada (28, 36, 40, 41, 42, 43), have demonstrated that heat treatment and storage of dried egg increase the blue fluorescence of a 10 per cent potassium chloride extract (26, 27) of the defatted powder. While Pearce (21) at first postulated that the active fluorescing materials in such extracts are hydrolytic products of the proteins, it appears much more likely that they arise by interaction of the proteins with reducing sugars (1, 13, 20, 25, 33). Furthermore, although Pearce and Thistle (26) and Thistle *et al.* (35) concluded that loss of palatability of dried egg is correlated significantly with increase in fluorescence of the salt-soluble constituents, others (2, 7) have presented evidence indicating a much closer relation between flavor and fluorescence arising in the lipide fraction by reaction of lipide amines with aldehydes (5, 6). Fluorescence has been suggested as an index of storage deterioration of dehydrated pork, dried banana, dried parsnip, ration biscuits, and butter (22) and also for following development of rancidity in lard (10).

Only very meager data are available on the fluorescence of milk, dairy products or milk constituents. It is well known that normal fresh milk emits greenish-yellow fluorescence when irradiated with ultraviolet light (3, 30) and that this fluorescence is due principally to riboflavin (34, 44). Gerngross and Schultz (9) observed that irradiation of milk causes an alteration in fluorescence from yellow to blue, perhaps by conversion of riboflavin to lumichrome (17), although Raoul's (31) data do not substantiate this explanation. Radley (29), finding that severe heat treatment and roller drying of milk shifted its fluorescence to the blue, attributed this change also to a breakdown of riboflavin.

To date the only published quantitative data on the fluorescence of milk other than that due to riboflavin are those of Pearce (22, 23), who studied the salt-soluble fluorescent compounds of defatted² dry whole milk.

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² It is not clear in the original just what solvent was used to extract the fat or how complete an extraction was attained.

He found: (a) greater initial blue fluorescence in extracts from spray dried than in those from roller dried milks and (b) decided increases upon storage. Since the increase in fluorescence was only slightly correlated with loss of palatability, Pearce (22, 23, 24) has concluded that fluorescence is not a satisfactory index of palatability in milk powder.

The fluorescence of the lipides of milk has not been studied extensively. Morgan and MacLennan (19)³ found that the fluorescence of butter and butterfat is yellow in contrast to that of margarine, which is blue, and Pearce (22) has reported a decidedly greater blue fluorescence in a butter serum (in 10 per cent potassium chloride solution) from rancid than from fresh butter.⁴ This latter observation has been made the basis of a method for assessing deterioration in butter (11).

The fact that several proteins, including casein, exhibit fluorescence when illuminated with ultraviolet has been demonstrated by Reeder and Nelson (32) and also by Vlès (39).

The work reported in this paper represents an attempt to develop a method capable of distinguishing among blue fluorescing materials produced in milk by such reactions as (a) breakdown of riboflavin or other reactions producing soluble blue-fluorescing material (b) lipide-amine-aldehyde interaction, and (c) interaction of protein and sugar. It was hoped that such a method would prove of value in following the course of storage deteriorations of dry milk products and possibly might serve as an objective criterion of palatability changes.

METHOD

In attempting to segregate fluorescing compounds from the several possible sources, a standard empirical method was devised involving fractionation of the constituents of milk into four categories: (I) those soluble in 67 per cent acetone; (II) those insoluble in 67 per cent acetone but soluble in acetone-ether (20:80); (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride; and (IV) those insoluble in (I), (II), or (III). This method is applicable to fluid milk or to dry milk reconstituted to a fresh basis. For dry whole milk, a 4-g. sample may be reconstituted by shaking with 31 ml. of distilled water.

The fractionating procedure is as follows: Add two volumes of acetone to one volume of milk or reconstituted milk, mix thoroughly, and filter through 15 cm. paper (S. and S. no. 597) which previously has been extracted exhaustively with 67 per cent acetone (2 acetone + 1 water). Wash the precipitate on the filter with two successive 10-ml. portions of 67 per cent acetone and make the combined filtrate and washings up to 100 ml. with 67 per cent acetone. This constitutes extract I.

³ See also review by Déribéré (4).

⁴ It is not stated whether the sample exhibited hydrolytic or oxidative rancidity.

Grind the precipitated residue from the first extraction with 20 ml. of C.P. acetone, transfer to a 250-ml. Erlenmeyer flask stoppered with a foil-covered cork, shake mechanically for 10 minutes and filter through 15 cm. paper (S. and S. no. 597). Further extract by shaking mechanically for 10 minutes with each of two successive 40-ml. portions of anhydrous ether. The combined acetone-ether filtrates made up to 100 ml. with ether constitute extract II. Although the solvent extracts small amounts of fluorescing material from the filter paper, no appreciable error is introduced if the blank is filtered in the same manner as the sample.

Dry the remaining protein residue by exposure to air at room temperature for 1 hour. Shake a 1-g. sample of it with 25 ml. of 10 per cent potassium chloride solution for 10 minutes and filter, again using S. and S. no. 597, 15 cm. paper. Wash the residue with two successive 25-ml. portions of the 10 per cent potassium chloride solution and make up to 100 ml. with 10 per cent potassium chloride. This is extract III. The 10 per cent potassium chloride does not extract fluorescing materials from the paper.

Extracts prepared in this manner were crystal clear. It will be noted that extract I is essentially identical to the filtrate used by Hand (12) for determination of riboflavin. To determine riboflavin, prepare tubes as follows and measure their fluorescence with the Coleman photofluorometer, using filters *B-2* and *PC-2* (see Hoffer *et al.* (15)).

Reading *A*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. water

“ *B*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. riboflavin solution containing 1 γ /ml.

“ *C*—Same as *B* but with fluorescence quenched with approximately 20 mg. of sodium hydrosulfite.

Then :

$$\left(\frac{A-C}{B-A} \right) = \gamma \text{ riboflavin per ml. filtrate}$$

$$\left(\frac{A-C}{B-A} \right) \times \frac{100}{1000} \times \frac{100}{4} = \text{mg. riboflavin per 100 g. powder.}$$

Determine blue fluorescence in each extract with the Coleman photofluorometer using filters *B-1* and *PC-1*. Adjust the galvanometer to read 70 with a quinine sulfate solution containing 0.2 γ /ml. Make blank determinations and report results in terms of net galvanometer readings multiplied by any necessary dilution factor.

For fresh whole milk and fresh dry whole milk it usually was necessary to dilute an aliquot of the acetone extract with an equal volume of the 67 per cent acetone solvent in order to obtain a reading on the scale. The ether and potassium chloride extracts from these products gave readings on the scale without dilution. In many aged samples, considerable dilu-

tion of the acetone and potassium chloride extracts was necessary, but rarely was it found necessary to dilute the ether extracts. It appeared desirable to express all results on a common basis and consequently net fluorescence readings were multiplied by dilution factors to convert them to the basis of fluorescence intensity of the 100-ml. extracts. For example,

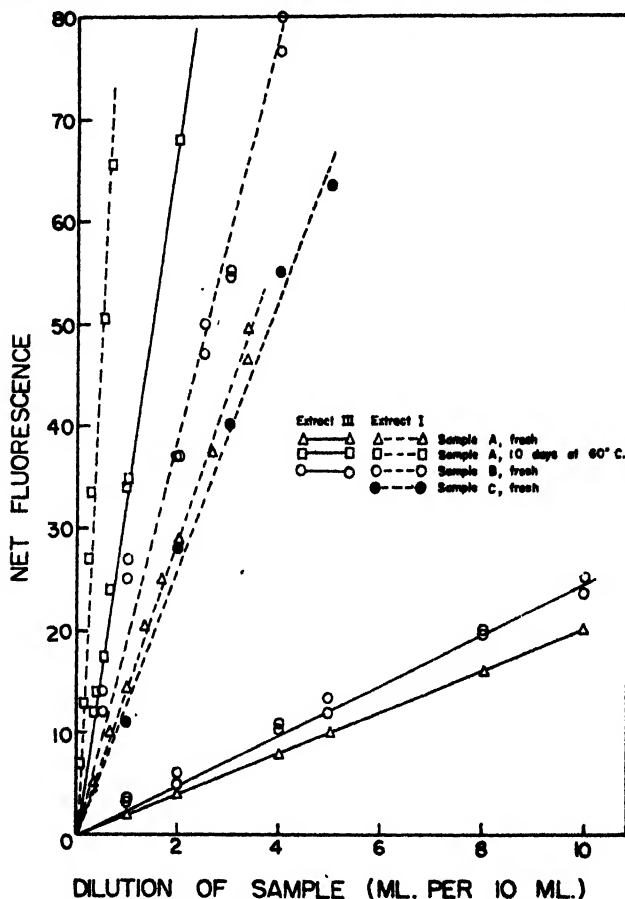


FIG. 1. Relation of fluorescence to dilution of extracts I and III. Indicated aliquots of the extracts were diluted to 10 ml. for fluorescence determination.

a sample diluted by ten times and reading 82.5 with a blank of 31.5 would have a net fluorescence of $(82.5 - 31.5) \times 10$ or 510. This procedure appears justified by the fact that over the range covered by the instrument the relation between net fluorescence and concentration of the acetone or potassium chloride extracts is linear and passes through the origin, as is illustrated in figure 1.

MATERIALS

1. *Dried milk* was prepared from University herd milk with the experimental drying equipment of the University of Minnesota.

2. *Casein* was prepared from fresh skim milk by the method of Van Slyke and Baker (38) as modified by Van Slyke (37), with the exception that the extraction with fat solvents was omitted. Calcium phosphocaseinate sols, used as a base for several series of simplified systems, were prepared by dispersing 100 g. of dry casein in 3 l. of saturated limewater, chilling in an ice bath and adding slowly with vigorous agitation 350 ml. of a solution containing 2.98 g. magnesium oxide, 34.47 g. potassium dihydrogen phosphate and 21.95 g. citric acid monohydrate per liter. During this back titration the following materials also were added: 1.014 g. potassium sulfate, 1.260 g. potassium carbonate, 5.600 g. sodium chloride, 1.264 g. potassium chloride, and 3.556 g. calcium hydroxide. The final pH of the sol was 6.7; it was very milky and reasonably stable.

3. *Milk serum protein* was prepared by removal of casein from skim milk with acetate buffer at pH 4.7, followed by exhaustive dialysis of the serum, concentration by pervaporation, freezing, and drying from the frozen state.

4. The *lactose* employed was of USP grade.

5. *Milk fat* usually was obtained by rendering butter and decanting and filtering the fat layer. For one experiment a sample of fat was extracted from whole milk by a macro-adaptation of the Roesse-Gottlieb method. Milk fat was incorporated into the simplified systems by emulsification with a hand homogenizer.

6. The *phospholipide-protein-complex* constituting the so-called "fat globule membrane" was obtained by concentrating and drying from the frozen state the buttermilk and butter serum obtained by churning cream washed by the method of Jenness and Palmer (16).

7. *Riboflavin* was obtained from the Eastman Kodak Company.

8. *Ascorbic acid* was obtained from Hoffmann-LaRoche, Inc.

EXPERIMENTAL

Partition of milk constituents among the various fractions. Some analyses of the several extracts were undertaken to ascertain how the constituents of milk are partitioned by the method used. All of these analyses were made on extracts from whole milk samples, six being dry and one liquid. The data are presented in table 1.

Extract I contains 36-43 per cent of the total dry matter of the 4-g. sample. The largest component of this dry matter is lactose; in fact, nearly all of the lactose of the sample appears in this extract. Undoubtedly the failure to recover the lactose quantitatively in extract I is attributable to incomplete washing of the sugar from the precipitate. The lactose de-

terminations were made by evaporating an aliquot of the extract nearly to dryness, making up a definite volume with zinc sulfate and sodium hydroxide solutions according to McDowell (18), filtering and determining lactose in the filtrate by the chloramine-T method of Hinton and Macara (14).

Some nitrogenous material is present in extract I. The amounts of 15.5 to 18.6 mg. per 100 ml. of extract correspond to 46.5–55.8 mg. per 100 g. of milk (12 per cent solids) and hence are somewhat greater than the usual non-protein nitrogen content of milk. Of course extract I also

TABLE 1
Analyses of extracts

Sample	Extract I			Extract II		Extract III
	Dry matter	Nitrogen	Lactose	Dry matter	Lipide P	Protein ^a
	(% of sample)	(mg./100 ml.)	(% of sample)	(% of sample)	(mg./100 g. fat)	(mg./100 ml.)
Dry whole milk						
1	.	.	.	27.0	4.12	
2	.	.	.	28.1	4.95	
3	40.2	18.2	.	25.6		74.0
4	42.7	18.6	.	25.8		72.3
5a	36.4	15.5	29.8 ^b	29.8 ^c	3.10 ^c	62.0
b			.	28.0		85.7
c				30.6		54.4
6	39.4	.	31.2 ^b	26.1		
Liquid whole milk						
7 ^d	.	.	.	25.5	2.55	83.0

^a Calculated as total nitrogen $\times 6.38$.

^b Lactose content of dry milk determined directly was 32.5 and 35.6% for samples 5a and 6, respectively.

^c Mojonmier extract of sample 5a yielded 31.2% fat and 27.7 mg. lipide P/100 g. fat.

^d Calculations made on basis of solids in sample.

contains riboflavin and undoubtedly a number of other minor soluble constituents. It contains very little, if any, fat.

Extract II contains the bulk of the fat of the sample. The amount of fat extracted in this way falls somewhat short of that extracted by the Mojonmier method. The amount of lipide phosphorus in this extract is only a small fraction of that present in the milk and extractable by the Mojonmier method.

The amount of dry matter extracted from 1 g. of defatted protein by the 10 per cent potassium chloride treatment amounts to 175 to 200 mg. The amount of nitrogen extracted by this treatment is equivalent to 54 to 86 mg. of protein. Actually only a portion of this nitrogen represents protein, since it was found that for sample 5c, at least, only approximately

80 per cent of the nitrogen was nondialyzable. Since this extract contains no fat, the non-nitrogenous portion must be composed of minerals and possibly lactose.

The residue that is not dispersed by 10 per cent potassium chloride undoubtedly is largely protein.

Fluorescence of milk constituents. The fluorescent characteristics of several milk constituents were studied using the *B-1* and *PC-1* filters in the Coleman instrument. The plot of fluorescence of riboflavin solutions as a function of concentration in figure 2 shows a linear relationship. It is

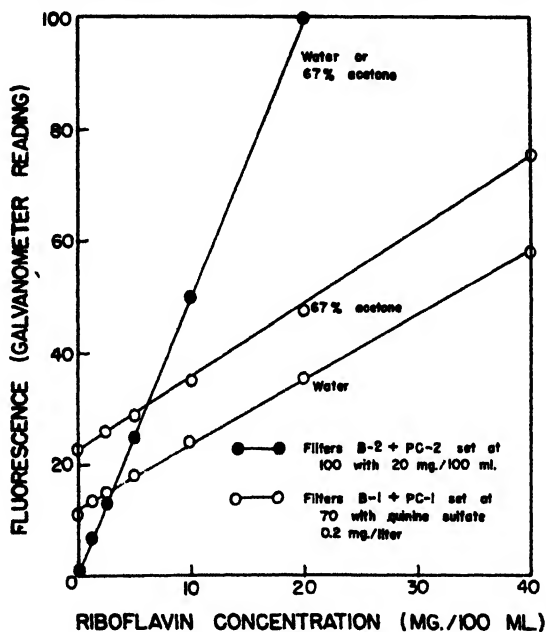


FIG. 2. Fluorescence of riboflavin as a function of concentration.

evident that considerable of the fluorescence of riboflavin is transmitted by filter *PC-1* and consequently that riboflavin accounts for a large portion of the fluorescence of extract I.

In figure 3 the fluorescence of several milk fat solutions is plotted as a function of concentration. The relation is approximately linear. Fat obtained by Roese-Gottlieb extraction exhibits a much higher fluorescence than that prepared by churning and rendering butter. This comparison between churned and Roese-Gottlieb extracted fat had to be made with ether as solvent, because the Roese-Gottlieb extracted fat was not completely soluble in the 20:80 acetone-ether mixture. Undoubtedly this phenomenon, as well as the higher fluorescence of the Roese-Gottlieb extracted fat, is due to the presence of phospholipides.

The fat obtained in extract II from whole milk exhibits a *net* fluorescence intermediate between those of the Roese-Gottlieb extracted fats and the churned fats, which is in accord with the previously established fact that extract II contains a portion but not all of the phospholipide of the milk.

The data in table 2 show that both casein and milk serum protein sols in phosphate buffer exhibit blue fluorescence but that the latter fluoresces much more intensely. The fluorescence of milk serum protein preparations may vary considerably, but the fluorescence exhibited by a given

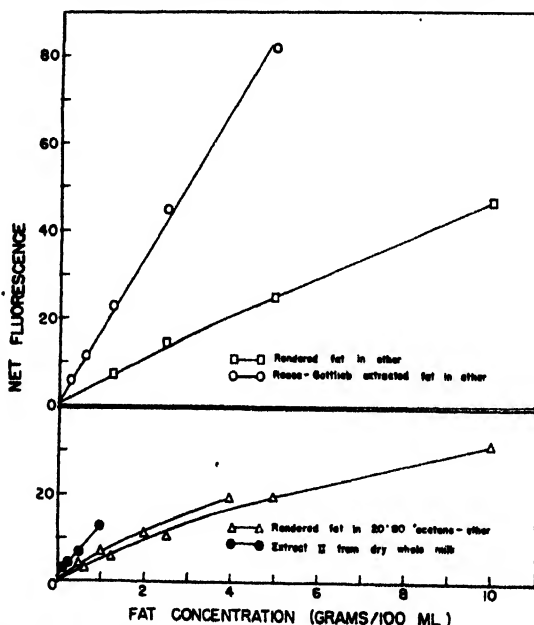


FIG. 3. Comparison of fluorescence of rendered butterfat, extracted fat, and extract II as a function of concentration. Fluorescence of the ether blank was 5.0; that of the 20:80 acetone-ether was 12.0.

preparation in phosphate buffer is approximately identical to that in 10 per cent potassium chloride solution at the same concentration. Casein alone is only slightly dispersible in 10 per cent potassium chloride, but the portion that does dissolve exhibits a considerable fluorescence.

Extraction of the defatted protein fraction of milk by the regular treatment with 10 per cent potassium chloride results in dispersion of only 54–86 mg. of protein from a 1-g. sample, as may be seen in table 1. Furthermore, it was found that grinding of this material with the 10 per cent potassium chloride solvent is without effect on amount of fluorescent material extracted. Variations in the amount of protein extracted do not

TABLE 2
Fluorescence of milk protein sols

Casein sols			Milk serum protein sols		
Sample	Conc.	Net fluorescence	Sample	Conc.	Net fluorescence
	(g./100 ml.)			(g./100 ml.)	
In phosphate at pH 6.6			In phosphate at pH 6.9		
1	0.94	19.0	1	0.54	23.0
2	0.47	10.0	2a	0.78	88.0
3	0.23	5.5	2b	0.39	48.5
			2c	0.20	25.0
Fraction soluble in 10% KCl			In 10% KCl		
Regular ^a	0.0063	5.0	2a	0.77	Too high
1 grinding ^b	0.0207	17.5	2b	0.38	49.5
3 grindings ^b	0.0344	21.0	2c	0.19	24.0

^a 1-g. samples of casein treated by the method described herein for preparing extract III.

^b Grinding of casein samples with 10% KCl was used in addition to the shaking employed in the regular method.

appear to be closely related either to the treatment of the product or to the fluorescence of the extract.

Solutions of lactose in water or in phosphate buffer at pH 6.6 and $\mu = 0.1$ were found to fluoresce only negligibly more than the blank.

Fluorescence of systems of milk constituents. In order to obtain further information on the contributions of the various milk constituents to

TABLE 3
Fluorescence of simplified systems of milk constituents

System	Constituents ^a	Net fluorescence							
		Extract I		Extract II		Extract III			
		B ^b	C	B	C	A	B	C	
		(per 4 g. complete system)				(per g. protein)			
1	Phosphocaseinate		10.1					8	
2	1 + lactose	10.1	16.0	3.8	1.7	3	3	7	
3	2 + serum prot.	15.3	25.8	3.5	3.7	4	4	16	
4	2 + fat	9.4	17.7	8.5	9.3	3	3	5	
5	4 + F.G.M. ^c	17.0	20.7	10.4	9.4	4	5	5	
6	5 + serum prot.	24.0	32.0	11.0	10.0	8	6	17	
7	6 + riboflavin	51.0	70.0	10.0	10.0	6	6	15	
8	7 + ascorbic	51.0		10.0		8	6		
Usual value for fresh dry whole milk		100.0		11.0		12.0			

^a Ratio of constituents was as follows: 1.00 casein : 2.15 lactose : 0.30 serum protein : 1.52 fat : 0.04 F.G.M. : 0.000075 riboflavin : 0.0010 ascorbic acid.

^b Letters designate replicate series.

^c Fat globule "membrane".

the fluorescence of the several extracts, the standard method described in this paper was applied to a series of simplified systems of milk constituents, each of which was dried from the frozen state.

Table 3 gives the data for fluorescence of these systems, while table 4 gives the contribution of each constituent to the fluorescence. For extracts I and II, fluorescence has been calculated on the basis of 4 g. of the complete system (*i.e.*, the system containing all of the constituents), while for extract III the data are expressed on the basis of 1 g. of protein taken for extraction. The fluorescence of extract I from the system containing all of the constituents mentioned approaches but does not attain that obtained from whole milk. Riboflavin evidently is the major contributor to the fluorescence of extract I, although smaller increments of fluorescent

TABLE 4

Contributions of constituents to fluorescence of simplified systems of milk constituents

Constituent	Contribution to fluorescence						
	Extract I		Extract II		Extract III		
	B	C	B	C	A	B	C
	<i>(per 4 g. complete system)</i>				<i>(per g. protein)</i>		
Caseinate		10.1					8
Caseinate + lactose ..	10.1	16.0	3.8	1.7	3	3	7
Serum protein ^a . . .	4.2	9.8	- 0.3	2.0	1	1	9
Serum protein	7.0	11.3	0.6	0.6	4	1	12
Milk fat	- 0.7	1.7	4.7	7.6	0	0	- 2
F.G.M.	7.6	3.0	1.9	0.1	1	2	0
Riboflavin	27.0	38.0	- 1.0	0.0	- 2	0	- 2
Ascorbic	0.0		0.0	..	2	0	

^a First value given for serum protein is computed as difference between systems 2 and 3, the second as difference between systems 5 and 6.

materials are extracted from the proteins. The serum protein preparation used in series *C* evidently carries more fluorescing materials than that used in series *B*.

Tables 3 and 4 show that the fluorescence of extract II is due principally to the lipides and that the characteristic fluorescence of whole milk extracts is satisfactorily reproduced in systems containing milk fat and fat globule "membrane". The proteins or materials associated with them in the defatted residue are responsible for the fluorescence extractable by 10 per cent potassium chloride from that residue. As expected, fat and riboflavin contribute nothing to the fluorescence of this extract.

Fluorescence of whole milk and the influence of processing. The method described herein was applied to 32-ml. samples of liquid whole milk, skim milk and butter milk, all from the same lot, and also to 4-g. samples of dry whole milk, dry skim milk, and dry buttermilk from a second lot. Table 5 indicates that the fluorescence of extract I from these products is approximately equal when expressed on the basis of fat-free solids in the

TABLE 5

Comparison of fluorescence of extracts from whole milk, skim milk, and buttermilk

Sample	Riboflavin	Net fluorescence			
		Extract I		Extract II	Extract III
	(mg./100 g. solids)	(per 4 g. solids)	(per 4 g. fat-free solids)	(per 4 g. solids)	(per g. defatted protein)
<i>Series I—Liquid</i>					
Whole milk	1.33	108	150	10	11.0
Skim milk	2.00	157	157	14	9.0
Buttermilk	1.67	147	147	25	17.0
<i>Series II—Dry</i>					
Whole milk	1.14	100	139	13	14.5
Skim milk	1.47	130	130	7	18.0
Buttermilk	1.59	146	146	20	22.2

sample. Buttermilk yielded the most fluorescing materials in extract II, probably because of its higher phospholipide content, but the reason for the high fluorescence of extract III from buttermilk is not apparent. Furthermore, the authors are unable to account for the anomalous relations exhibited by extracts II and III of the skim milk samples in that the liquid and dry samples differ considerably.

The effects of variations in pasteurization temperature, of condensing, and of spray drying were studied on a lot of mixed whole milk. The sample size was adjusted to furnish 4 g. of solids in each case. The results, shown in table 6, indicate that heat treatment in the range of 145 to 195° F.

TABLE 6

Effect of heat treatment, condensing and drying on fluorescence of extracts

Sample	Pasteurization temp. for 30 min.	Riboflavin	Net fluorescence		
			Extract I	Extract II	Extract III
	(° F.)	(mg./100 g. solids)	(per 4 g. solids)		(per g. defatted protein)
Whole milk	Raw	0.99	108	12	10.5
" "	145	1.05	116	12	9.5
" "	155	1.05	116	11	11.5
" "	165	1.09	114	14	12.5
" "	175	1.10	124	14	13.0
" "	185	1.10	125	13	13.0
" "	195	1.15	132	13	14.5
Condensed	165	1.22	138	12	9.5
Spray dried	165	1.10	120	12	15.0
Spray dried	150		87	12	7.4
Normal	185		95	12	7.4
Spray dried	150		94	12	10.1
High temp.	185		93	13	7.4

for 30 minutes has some effect in increasing fluorescence in extract I, possibly some in extract III, but none in extract II. The effects of condensing and spray drying, if any, do not appear to be very marked.

In another experiment, also recorded in table 6, in which pasteurizing treatments of 150 and 185° F. were combined with normal and high temperature drying conditions, no significant effects of either treatment on fluorescence were noted.

DISCUSSION

While the procedure adopted for fractionating the constituents of milk is rather empirical, it is felt that a reasonable approach has been made to determining which of those constituents are capable of emitting blue fluorescence when illuminated with ultraviolet. Furthermore, it is considered that the scheme which has been evolved may prove of use in following changes occurring during storage of dry whole milk.

Riboflavin appears to be the principal fluorescent material of extract I but very evidently certain other materials also are involved. In the simplified systems some of the "non-riboflavin fluorescence" was contributed by the protein preparations used, but any speculation on the nature of the specific protein fraction that may be responsible for this effect is fruitless at present. Whole milk evidently contains fluorescent materials soluble in 67 per cent acetone other than those included in the simplified systems, because in no case did the most complete simplified system yield fluorescence in extract I equaling that of whole milk.

The fluorescence of extract II appears to depend, in part, on its phospholipide content. Evidently the major portion of these lipides is not extracted at all but remains in the final residue. Consequently, variations in the fluorescence of this extract might be expected to depend on the extent of extraction of phospholipides. The most satisfactory scheme would be one which extracted all of the phospholipides, but under the empirical conditions used, the proportion of phospholipide extracted probably is sufficiently constant so that no great variations in fluorescence are introduced from that cause.

Ten per cent potassium chloride disperses a fraction of milk protein which is associated with a certain amount of fluorescing ability. No exhaustive attempt was made to determine just which protein fraction is involved. However, the data secured on fluorescence of milk proteins in 10 per cent potassium chloride indicate that both casein and at least some of the serum protein fractions are involved. Here again it might be argued that the ideal situation would be to disperse all of the protein in extract III, leaving no undispersed residue, but to date it has not proved possible to prepare such a dispersion that is satisfactory for fluorescence measurements.

The data fail to indicate any pronounced change in fluorescence proper-

ties during the processing involved in manufacture of dry whole milk. The procedure employed in this study is being applied to following the changes occurring during heat treatment of milk and storage of dry whole milk.

SUMMARY AND CONCLUSIONS

A method is presented for evaluating the fluorescence characteristics of milk by fractionating the constituents of milk into (I) those materials soluble in 67 per cent acetone, (II) those insoluble in (I) but soluble in 20:80 acetone-ether, (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride, and (IV) those insoluble in (I), (II) or (III), and determining the blue fluorescence of the solutions of (I), (II) and (III).

As might be expected, riboflavin is the largest contributor to the fluorescence of extract I, although proteins and probably other constituents also contribute. The lipides, particularly the phospholipides, are the fluorescent compounds of extract II, while the proteins contribute the fluorescent materials dispersible in 10 per cent potassium chloride.

The normal processing of dry whole milk appears to be without effect on the fluorescence of these extracts.

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THE RELATIONSHIP OF THE CHANGE IN pH EFFECTED BY INCUBATION TO OTHER SEMEN CHARACTERISTICS¹

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A number of simple, rapid tests have been applied to the evaluation of semen, although no single measure is presently recognized as an adequate index of fertilizing capacity. The reports of several workers (1, 2, 4, 5, 8, 15, 17) would indicate that the initial pH of semen may be related to semen quality and/or used as a criterion of relative fertility. Significant correlations have been found between the initial pH and spermatozoa concentration (1, 2, 3, 9, 14, 15, 17), ejaculate volume (1, 2, 9, 17), spermatozoa motility (1, 2, 6, 9, 11, 17), sugar content (5) and buffer capacity (4) of semen, and the glucose loss, lactic acid gain and viability following incubation at 46.5° C. for 1 hour (17). The initial pH was inversely related to these semen characteristics. However, Swanson and Herman (16) found no appreciable relationship between the pH of fresh semen and conception rate.

Although the initial pH was found to be helpful in the evaluation of semen in a number of studies, several reports would indicate that the final pH and/or the change in pH effected by incubation and/or storage of semen provides a more satisfactory index (6, 11, 13, 19, 20). The final pH of semen following incubation at 37° C. and storage at 40° F. was correlated with the concentration, motility, oxygen consumption and fertility of spermatozoa (13) and the conception rate (11), respectively. Although Anderson (4) found no significant correlation between the change in pH upon incubating semen at 37° C. for 1 hour and the buffer capacity or specific gravity of semen, a high correlation was found between the change in pH and the spermatozoa concentration (6, 13), initial motility (6, 13), oxygen consumption (13) and fertility (13, 19, 20, 21) of spermatozoa. Measuring the drop in pH of semen stored at 27 to 29° C. and at 10° C., Dougherty and Ewalt (10) found the decrease in pH to be related to the motility and viability of spermatozoa. These workers showed that refrigeration and chemical inactivation, both of which inhibit motility, also prevent rapid decreases in pH. Several workers (6, 13) believe that the change in pH of semen is a reflection of the general metabolism of spermatozoa involving the number and activity of spermatozoa, respiration and glycolysis. For development of the maximum increase in acidity during

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incubation, Anderson (6) suggested the necessity of a high concentration of actively motile spermatozoa, an adequate quantity of glucose or other reducible sugar, and conditions favorable for the retention in the semen of acid products, *i.e.*, anaerobic conditions.

The present study was initiated when it was observed that, of a large number of semen quality tests employed in this laboratory, the change in pH during incubation seemed to be giving the best over-all estimate of semen quality. Since most of the data recorded in the literature represent a relatively limited number of ejaculates and since the relationships of the pH change to only a few semen characteristics are reported, the purpose of this investigation was to contribute additional data to the subject and to determine the value of the change in pH effected by incubation as an index of semen quality.

EXPERIMENTAL PROCEDURE

The relationship of the change in pH upon incubation of semen at 37° C. for 1 hour to concentration, initial motility, and viability of spermatozoa and percentage of abnormal spermatozoa, was studied in 203 semen specimens from 11 bulls. The average sample consisted of 2.33 ejaculates (range, 1-5 ejaculates).

Soon after the semen was ejaculated, the initial motility was estimated by using a constant temperature stage incubator adjusted to 100° F., the initial pH was measured with a Beckman glass electrode potentiometer, and an aliquot of each sample was incubated at 37° C. for 1 hour, after which time the pH was measured again. The spermatozoa concentration was determined with a cytometer, and the percentage of morphologically abnormal spermatozoa was estimated in fixed and stained semen smears. The initial motility ratings used in this study represent gradations of 1 to 20 units and are approximately equivalent to per cent motility divided by five. Viability was measured as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

RESULTS AND DISCUSSION

The pertinent data obtained in this study are shown in table 1. Highly significant positive correlations were found between the pH change of incubated semen and each of the characteristics, of concentration, initial motility, and viability of spermatozoa, whereas a significant negative correlation existed between the pH drop and the percentage of morphologically abnormal spermatozoa. Of these semen characteristics, the concentration and initial motility of spermatozoa showed the highest degree of relationship to the decrease in pH. These correlation coefficients (0.460 and 0.436, respectively) are remarkably similar to those reported by Anderson (6) (0.476 and 0.450, respectively). From a casual observation, these coeffi-

TABLE 1
Correlation coefficients showing interrelationships of various semen characteristics

Semen characteristics	1 pH change	2 Viability	3 Spermatozoa concentration	4 Initial motility	5 Abnormal spermatozoa
1 pH change					
2 Viability		0.2062 ± 0.0675**			
3 Spermatozoa concentration			0.4600 ± 0.0556**		
4 Initial motility			0.2639 ± 0.0656**	0.4364 ± 0.0571**	
5 Abnormal spermatozoa				0.2277 ± 0.0669**	
Means ± standard error	0.336 ± 0.016 ^a	35.29 ± 2.32 ^b	0.903 ± 0.023 ^c	13.1 ± 0.345 ^d	
Range	0.0-1.84	0.0-128 ^e	$R_{1,2345} = 0.5658 \pm 0.0484$		0.1-19.8
					2.0-43.0

^a Units of pH.

^b Percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

^c Millions per mm.³

^d Motility value × 5 is approximately equivalent to per cent motility.

^e Per cent morphologically abnormal.

^f Three values found above 100%; 12 values above 88%.

** Highly significant.

cients may appear to be low. However, they were calculated from the ungrouped data of individual semen samples.

The multiple correlation coefficient (0.5658 ± 0.0484) between the pH change and the four semen characteristics studied was highly significant. The pH change of incubated semen would seem to have great potential worth in the evaluation of semen, since it is related to a number of different characteristics.

It long has been known that lactic acid formation occurs during the storage of semen and is accompanied by a decrease in the quantity of glucose or other reducing sugars, a reduction in motility and an increase in acidity (18). The incubation of semen increases the rate of these reactions, allowing measurable differences in the end-products within short periods of time. Since the rate and the extent of the decrease in pH were found to be proportional to both motility and concentration of spermatozoa in this study as well as in others (6, 13), the pH drop effected by incubation appeared to be a quantitative reaction dependent upon the metabolic activity of the individual spermatozoan and the total number of spermatozoa present. This provided an indirect measure of the over-all metabolism of semen. That the effects of the percentage of morphologically abnormal spermatozoa reflect upon the change in pH of a semen specimen is indicated by the inverse relationship between these two characteristics (correlation coefficient = -0.324). This phenomenon, in which a large number of morphologically abnormal spermatozoa minimized the extent to which pH was changed, would suggest that these spermatozoa are participating in katabolism either very little or not at all. Since deformed spermatozoa tend to impede the motility of the more normal cells, they perhaps further inhibit the rate of metabolism of the total specimen, producing less lactic acid and other acid products, thereby resulting in a lesser degree of pH drop. Table 1 shows a highly significant inverse relationship between the percentage of morphologically abnormal spermatozoa and the initial motility. The abnormal spermatozoa commonly appear to survive the normal spermatozoa, as determined by the maintenance of motility. This observation would indicate the possibility of energy conservation in abnormal spermatozoa and would be in accord with the observations previously discussed. Some studies suggest that results of measures of spermatozoa metabolism, such as the rate of respiration (18) and the rate of glycolysis (7), provide the best single indications of fertility. However, the measures of oxygen consumption and products of glycolysis of spermatozoa are tedious, necessitate special apparatus and conditions restricted to well-equipped laboratories, and require personnel of specialized training, thereby limiting their field usage. A high degree of relationship exists between the pH change and oxygen consumption (13), a number of other semen characteristics and fertility (13, 19, 20, 21). It would seem that complex,

time consuming tests and the use of a large number of simple semen quality tests would be largely obviated by the use of the pH change upon incubation as a single measure of semen quality. Although a pH-meter was employed in this study, a series of indicators could be readily adapted to field use as suggested by Laing (13). Incubation of semen for 1 hour produces greater changes in semen pH than does incubation for 0.5 hour; however, some artificial breeding units may find the shorter incubation period more compatible with their semen collection schedule. Information provided by this test would be available for the inseminator when needed, *i.e.*, before dilution of the semen and before insemination of the cow.

SUMMARY

1. A study was made of the relationship of the decrease in pH during incubation of 203 semen specimens composed of 473 ejaculates from 11 bulls to other semen characteristics.

2. Highly significant coefficients of correlation found between the change in pH and other characteristics of semen were: concentration of spermatozoa, 0.46; initial motility of spermatozoa, 0.44; viability of spermatozoa, 0.21; and percentage of morphologically abnormal spermatozoa, -0.32. The multiple coefficient of correlation (0.57) between these four semen characteristics and the drop in pH was highly significant. These data suggest a significant relationship of the pH change in incubated semen to a number of different semen characteristics and indicate its value as an indirect measure of over-all semen metabolism.

3. The results of this study, in combination with those of other investigations involving fertility data, would seem to indicate that the change in pH effected by incubation is probably the best simple, quick test of semen quality available at the present time.

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ASSOCIATION ANNOUNCEMENTS
PROGRAM
FORTY-THIRD ANNUAL MEETING
OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION
UNIVERSITY OF GEORGIA
ATHENS, GEORGIA
JUNE 14-16, 1948
PROGRAM COMMITTEE

GENERAL:

FORDYCE ELY, *Chairman*
Ohio State University
JENNINGS B. FRYE
University of Georgia

EXTENSION:

E. H. LOVELAND, *Chairman*
University of Vermont
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University of Minnesota
D. V. JOSEPHSON
Ohio State University

PRODUCTION:

G. H. WISE, *Chairman*
Iowa State College
L. A. MOORE
Bureau of Dairy Industry
D. M. SEATH
University of Kentucky

REGISTRATION

SOULE HALL

UNIVERSITY OF GEORGIA

Meetings will be held in the buildings on the campus of the College of Agriculture of the University of Georgia. Headquarters will be in Soule Hall.

The opening session will be in Hardman Hall. All sectional meetings will be in Dawson Hall. Rooms there will be assigned based on the needs of the sections. The general business session on Wednesday afternoon will be in the Auditorium, Dawson Hall.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and 2" x 2" slides. Projectors for 16 mm. movies will be available by ar-

rangement. Please advise respective section chairmen by mail of the type of projection equipment, if any, which will be needed for the presentation of your paper.

COMMITTEE MEETINGS

Those wishing rooms for Extension, Production, and Manufacturing Section Committee meetings should write or contact Jennings B. Frye, Jr., Dairy Department, University of Georgia.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular sessions will please contact Jennings B. Frye, Jr., of the Dairy Department, University of Georgia. Provision can be made for a limited number of breakfasts, luncheons, or dinners for special groups.

EXTENSION EXHIBITS

Extension Dairymen desiring space for exhibition of material will please contact Frank W. Fitch, Extension Dairyman, University of Georgia. The exhibits will be in the Auditorium, Dawson Hall.

PROGRAM OF ENTERTAINMENT

(Principally for the Ladies)

Monday, June 14

10:00 A.M. TOUR—Antebellum Homes of Athens

3:00 P.M. TEA—Founders Memorial Gardens

Compliments of Landscape Architecture Department
and Georgia Feed Manufacturers Association

8:00 P.M.* ENTERTAINMENT—Physical Education Building

Early American Dances—The Atlanta Promenade Club

Negro Spirituals

Group Singing

Tuesday, June 15

MORNING FREE. Convenient busses to shopping district

12:30 P.M. LUNCHEON—Athens Country Club

Compliments of Kraft Foods Company

3:30 P.M. ART EXHIBIT AND DEMONSTRATION—Fine Arts Department

8:00 P.M.* RECEPTION AND DANCE—Memorial Hall

(Formal for Ladies)

Wednesday, June 16

3:00 P.M. BRIDGE—Physical Education Building

7:00 P.M.* BARBECUE—Amphitheatre

**8:30 P.M.* INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS—
Amphitheatre**

* These features open to all in attendance. Other events open to ladies only. Admission to all events by badge only.

GENERAL PROGRAM

Monday, June 14

Eastern Standard Time

9:30–12:00 OPENING SESSIONS, *Hardman Hall*

II. B. HENDERSON, *Dairy Department, University of Georgia, presiding*

Invocation

DR. PAUL C. HOWLE, *Pastor, First Christian Church, Athens*

Introduction of Officers and Guests

Address of Welcome

H. W. CALDWELL, *President, University of Georgia*

Presidential Address

PAUL H. TRACY, *President, American Dairy Science Association*

Intermission

Music

Guest Speaker

PAUL W. CHAPMAN, *Dean, College of Agriculture, University of Georgia*

Announcements

1:30– 4:30 SECTIONAL MEETINGS

Production Section A

**Genetics and Endocrine Investigations
*Dawson Hall***

Production Section B

**Type, Vitamins, Metabolism, Techniques
*Dawson Hall***

Manufacturing Section

**Dry Milk, Condensed Milk, Ice Cream, Cream
*Dawson Hall***

Extension Section

**Records and Interpretations
*Dawson Hall***

4:30- 5:30 COMMITTEE MEETINGS

8:00 ENTERTAINMENT, *Physical Education Building*
Early American Dances—Atlanta Promenade Club
Negro Spirituals
Group Singing

Tuesday, June 15

9:00-12:00 SECTIONAL MEETINGS**Production Section A**

Calf Problems

Dawson Hall

Production Section B

Artificial Breeding

Dawson Hall

Manufacturing Section

Pasteurization, Microbiology, Cheese

Dawson Hall

Extension Section

Teaching Methods and Exhibits

Dawson Hall

1:30- 4:00 SECTIONAL MEETINGS**Joint Meeting of Production and Extension Sections**

Dawson Hall

Manufacturing Section

Cheese

Dawson Hall

4:00- 5:30 PRODUCTION AND EXTENSION SECTIONS**Committee Reports**

MANUFACTURING SECTION

Business Meeting

8:00 RECEPTION AND DANCE, *Memorial Hall*
(Formal for Ladies)

Wednesday, June 16

9:00-11:00 SECTIONAL MEETINGS**Production Section A**

Parturient Changes in Blood and in Mammary Secretions

Dawson Hall

Production Section B

Forages, Hay
Dawson Hall

Manufacturing Section A

Chemistry
Dawson Hall

Manufacturing Section B

Homogenized Milk, Sanitation, Microbiology
Dawson Hall

Extension Section

4-H Clubs and Testing Rules
Dawson Hall

11:00-12:00 BUSINESS MEETINGS

Production Section, *Dawson Hall*

Manufacturing Section, *Dawson Hall*

Extension Section, *Dawson Hall*

1:00 GROUP PICTURE, *Amphitheatre*

1:30- 3:00 SECTIONAL MEETINGS

Production Section A

Feeding and Management
Dawson Hall

Production Section B

Forages, Pastures
Dawson Hall

Manufacturing Section

Dairy Sanitation Symposium
Dawson Hall

3:00- 5:00 GENERAL BUSINESS SESSION, *Auditorium, Dawson Hall*

7:00 BARBECUE, *Amphitheatre*

INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS

PROGRAM OF MANUFACTURING SECTION

Monday, June 14

Afternoon Session

1:30- 4:30 **DRY MILK, CONDENSED MILK, ICE CREAM, CREAM.** D. V. JOSEPHSON, *Chairman.*

M1 The Effect of the Addition of Ascorbic Acid to Milk on the Keeping Quality of Its Dried Product. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

- M2 The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*
- M3 The Effect of Heat Treatment on the Reducing Systems of Milk. S. T. COULTER, HERBERT HARLAND, AND ROBERT JENNESS, *University of Minnesota.*
- M3-a The Heat Treatment of Milk Necessary to Prevent Lipolytic Activity in Its Dried Product (A Preliminary Report). GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*
- M4 The Isolation of Compounds Responsible for the Stale Flavor Developed in Dried Whole Milk. I. The Distribution of Stale Flavor between the Fractions of Reconstituted Stale Whole Milk Powder. R. M. WHITNEY AND P. H. TRACY, *University of Illinois.*
- M5 A Solubility Method for the Determination of Alpha and Beta Lactose in Dry Products of Milk. R. P. CHOI, C. W. TATTER, AND B. W. FAIRBANKS, *American Dry Milk Institute, Inc., Chicago, Illinois.*
- M6 The Viscosity and Heat Stability of Milks Subjected to High Temperature Processing. B. H. WEBB AND C. F. HUFNAGEL, *Bureau of Dairy Industry, U.S.D.A.*

FIFTEEN MINUTE RECESS.

- M7 The Microbiological Keeping Quality of Bulk Condensed Milk. A. M. PEARSON AND F. E. NELSON, *Iowa State College.*
- M8 The Use of Sweetened Condensed Whole Milk in the Manufacture of Caramels. J. J. SHEURING AND P. H. TRACY, *University of Illinois.*
- M9 Influence of the Mineral Content of Water on the Properties of Ice Cream Mixes. ROBERT A. HIBBS AND W. A. KRIENKE, *University of Florida.*
- M10 Observations on the Effects of Various Stabilizing and Emulsifying Materials on the Properties of Ice Cream. W. S. ARBUCKLE, R. B. REDFERN, AND L. F. BLANTON, *North Carolina State College.*
- M11 The Effect of a Mannitol of Beef Fat on the Whipping Qualities, Body and Texture of Ice Cream. RALPH NADEN, J. J. SHEURING AND P. H. TRACY, *University of Illinois.*

- M12 A Study of the Fat Emulsion in Natural and Artificial Creams. W. E. SNYDER, *University of Georgia*, AND H. H. SOMMER, *University of Wisconsin*.

4:30- 5:30 **COMMITTEE MEETINGS.**

Tuesday, June 15

Morning Session

- 9:00-12:00 **PASTEURIZATION, MICROBIOLOGY, CHEESE.**
E. M. BARKER, *Chairman*.
- M13 Preservation of Milk for the Phosphatase Test. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*
- M14 Differentiation of Microbial Phosphatases from Milk Phosphatase. RALPH P. TITSTLER, OSCAR S. SAGER, AND GEORGE P. SANDERS, *Bureau of Dairy Industry, U.S.D.A.*
- M15 A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. JOHN HETRICK, *Dean Milk Co., Rockford, Illinois*, AND P. H. TRACY, *University of Illinois*.
- M16 Isolation of Heat-induced Flavor Compounds from Milk. STUART PATTON AND DONALD V. JOSEPHSON, *Ohio State University*.
- M17 Some Observations on the Efficiency of High-temperature Short-time Pasteurization of Chocolate Milk. MARVIN L. SPECK AND CHARLES D. COLVARD, *North Carolina State College*.
- M18 Use of the Direct Microscopic Method for Pasteurized Dairy Products. M. J. PRUCHA AND VIRGINIA FRAZEE, *University of Illinois*.
- M19 Bacteriophage Production by Cultures of *Streptococcus lactis*. F. J. BABEL, *Purdue University*.
- M20 Electron Microscope Studies of Bacteriophages Active against *Streptococcus lactis*. C. E. PARMELEE, P. H. CARR, AND F. E. NELSON, *Iowa State College*.
- M21 Some Factors Affecting the Rate of Acid Production by Cheese Cultures. H. C. OLSON AND FRANCIS D. COHENOUR, *Oklahoma A. and M. College*.
- M22 Methods of Controlling the pH of Fermenting Dairy Products and the Effects of pH Control. WAYNE I. TRETSVEN, *Chicago, Illinois*.

- M23 Chemical Changes Occurring in Limburger Cheese during Accelerated Ripening. W. K. STONE AND S. L. TUCKEY, *University of Illinois*.
- M24 A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-frequency Heat. F. V. KOSIKOWSKY, B. L. HERRINGTON, AND A. C. DAHLBERG, *Cornell University*.
- M25 Increasing Efficiency and Reducing Costs in the Manufacture of Cheddar Cheese. D. M. IRVINE AND WALTER V. PRICE, *University of Wisconsin*.

Tuesday, June 15

Afternoon Session

1:30- 4:00 **CHEESE.** P. R. ELLIKER, *Chairman*.

- M26 The Use of Nonfat Dry Milk Solids in the Manufacture of Cheddar Cheese from High Fat Content Milk. G. H. WILSTER AND C. E. JOHNSON, *Oregon State College*.
- M27 The Problem of Sampling Cheddar Cheese for Analysis. WILLIAM C. WINDER AND WALTER V. PRICE, *University of Wisconsin*.
- M28 The Influence of *Oospora lactis* in Promoting Changes in the Constants of Cheese Fat during Ripening of Cheddar Cheese. S. L. TUCKEY, W. O. NELSON, AND R. V. HUSSONG, *University of Illinois*.
- M29 Studies of Sources of the Typical Flavor in Cheddar Cheese. HAROLD E. CALBERT AND WALTER V. PRICE, *University of Wisconsin*.
- M30 The Influence of Temperature of Ripening on the Concentration of Tyramine in American Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, *Cornell University*.
- M31 Methods for Studying the Ripening of Cheese. H. H. SOMMER AND W. J. HARPER, *University of Wisconsin*.
- M32 The Effect of Added Amino Acids on Flavor Development in Cheddar Cheese. R. J. BAKER AND F. E. NELSON, *Iowa State College*.
- M33 Studies of Amino Acids in Cheddar Cheese during Ripening. W. J. HARPER AND A. M. SWANSON, *University of Wisconsin*.
- M34 The Influence of Various Lactobacilli and Certain Streptococci on the Chemical Changes, Flavor Develop-

ment, and Quality of Cheddar Cheese. R. P. TITSLER, GEORGE P. SANDERS, H. R. LOCHRY, AND O. S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

4:00- 5:00 BUSINESS MEETING.

Wednesday, June 16

Morning Session

9:00-11:00 SECTION A, CHEMISTRY. D. V. JOSEPHSON, *Chairman.*

M35 Some Physiological Effects of Dietary Lactose. JESSIE ELIZABETH FISCHER AND T. S. SUTTON, *Ohio State University.*

M36 The Limitations of the Refractometer Readings of Milk Serums in Detecting Watered Milk. L. R. ARRINGTON AND W. L. FOUTS, *University of Florida.*

M37 The Application of Flame Photometry to Determinations of Calcium, Potassium and Sodium in Milk. W. A. KRIENKE AND NATIAN GAMMON, *University of Florida.*

M38 A Rapid Method for the Determination of Nitrogen in Milk Products by Direct Nesslerization of the Digested Sample. J. H. HETRICK, *Dean Milk Company, Rockford, Illinois,* AND R. M. WHITNEY, *University of Illinois.*

M39 Studies on Separation and Fractionation of Casein. E. C. HAGBERG AND A. M. SWANSON, *University of Wisconsin.*

M40 The Fractionation of Milk Fat by Molecular Distillation. E. L. JACK AND MRS. L. B. OLSEN, *University of California.*

M41 The Measurement of Free Fatty Acids in Dairy Products. H. A. HOLLENDER AND H. H. SOMMER, *University of Wisconsin.*

M42 The Determination of Linoleic Acid in Milk Fat. P. S. SCHAFER AND GEORGE E. HOLM, *Bureau of Dairy Industry, U.S.D.A.*

M43 Retention of Ascorbic Acid, Changes in Oxidation-reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*

M44 The Effects of the Treatment of Milk and the Subsequent Storage of Cream and Butter below Freezing

Temperatures upon the Sensitivity of Fat to Oxidative Deterioration as Determined by the Re-emulsification Test. VLADIMIR N. KRUKOVSKY, E. S. GUTHRIE AND FRANK WHITING, *Cornell University*.

- M45 Ascorbic Acid Oxidation in Milk by Preformed H_2O_2 . VLADIMIR N. KRUKOVSKY, *Cornell University*.

Wednesday, June 16

Morning Session

9:00-11:00 SECTION B, **HOMOGENIZED MILK, SANITATION, MICROBIOLOGY.** E. M. BARKER, *Chairman*.

- M46 Stimulation of the Oxidized Flavors in Homogenized Milk by Light as Governed by the Vitamin C Content of the Milk. E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY, *Cornell University*.
- M47 Studies on Seepage from Bottles of Homogenized Milk. E. O. HERREID, *University of Illinois*.
- M48 The Leucocyte Count of the Complete Milking of Normal Animals for Complete Lactation Periods. E. O. ANDERSON, *University of Connecticut*.
- M49 Effect of Some Water Constituents on Quarternaries. W. S. MUELLER AND D. B. SEELEY, *University of Massachusetts*.
- M50 Germicidal Effectiveness of Certain Hypochlorides and Quaternary Ammonium Compounds under Simulated Plant Conditions. P. R. ELLIKER, *Oregon State College*, AND K. R. SPURGEON, *Purdue University*.
- M51 Sanitizing Milk Cans in Mechanical Can Washers. G. W. REINBOLD, S. L. TUCKEY, R. V. HUSSONG, AND P. H. TRACY, *University of Illinois*.
- M52 Some Factors Involved in Developing a Sediment Test for One-pint Samples of Cream Taken Off the Bottom of the Container. RAYMOND W. MYKLEBY AND BEN M. ZAKARIASEN, *Land-O-Lakes Creameries, Inc., Minneapolis, Minnesota*.
- M53 A Skunk-like Odor of Bacterial Origin in Farm-separated Cream. T. J. CLAYDON, *Kansas State College*.
- M54 Coliform Bacteria in Butter. R. N. SINGH AND F. E. NELSON, *Iowa State College*.

- M55 The Effect of *Streptococcus lactis* and Coliform Organisms on Soluble Nitrogen in Milk. E. B. COLLINS AND F. E. NELSON, *Iowa State College*.

11:00-12:00 BUSINESS MEETING.

Wednesday, June 16

Afternoon Session

- 1:30- 3:00 SYMPOSIUM ON ASPECTS OF SANITATION IN THE DAIRY INDUSTRY.** P. R. ELLIKER, *Chairman*. K. G. WECKEL, *Leader*.
Chemical and Physical Aspects of Cleaning Dairy Equipment. H. G. HARDING AND H. A. TREBLER, *National Dairy Research Laboratories, Inc., Baltimore, Maryland*.
Aspects of Quarternary Compounds. LUTHER BLACK, *U. S. Public Health Service, Cincinnati, Ohio*.
Bacteriophage and Its Relation to Sanitary Practices. F. J. RABEL, *Purdue University*.
- 3:00- 5:00 GENERAL BUSINESS SESSION, Auditorium, Dawson Hall.**
- 7:00 BARBECUE, Amphitheatre.**

PROGRAM OF PRODUCTION SECTION

Monday, June 14

Afternoon Session

- 1:30- 4:30 SECTION A, GENETICS AND ENDOCRINE INVESTIGATIONS.** G. H. WISE, *Chairman*.
- P1** The Relative Merits of a Cow's Own Record and Her Progeny Test for Predicting the Butterfat Production of Her Future Daughters. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University*.
- P2** Preliminary Results from the Crossing of Two Inbred Lines of Holsteins on Growth and Milk Production. N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, *University of California*.
- P3** Genetic Variation in the Levels of Blood Plasma Carotene and Vitamin A in Dairy Cattle. R. E. MATHER, *New Jersey Agricultural Experiment Station*.
- P4** Measurement of the Rate of Endocrine Gland Secretion as a Tool in the Genetic Selection of Dairy Cattle. C. W. TURNER, *University of Missouri*.

- P5 Thyroid Secretion Rate and Its Relation to Various Physiological Processes. VICTOR HURST, *University of Missouri*.
- P6 The Effect of Low Levels of Thyroprotein Feeding upon Milk and Milk Fat Production, Body Weight, Body Temperature, Heart Rate and Respiration Rate of Dairy Cows. R. G. SWANSON AND C. B. KNOTT, *Pennsylvania State College*.
- P7 Effects of Feeding Thyroprotein to Milking Cows in Summer. K. E. GARDNER AND T. W. MILLEN, *University of Illinois*.
- P8 Effects of Feeding Thyroprotein during Successive Lactations. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
- P9 Factors Controlling the Extent of Duct Growth in Mammary Glands. I. The Influence of an Estrogen in a Hereford Heifer. RALPH P. REECE, *New Jersey Agricultural Experiment Station*.
- P10 The Value of Oxytocin for Reducing Fluctuations in Milk and Fat Yield during Experimental Periods. H. P. ADAMS AND N. N. ALLEN, *University of Wisconsin*.
- P11 The Role of Certain Hormones in Spermatogenesis. J. D. SAMPATH KUMARAN, *University of Missouri*.

Monday, June 14
Afternoon Session

1:30- 4:30 SECTION B, TYPE, VITAMINS, METABOLISM, TECHNIQUES. L. A. MOORE, *Chairman*.

- P12 The Relationship between Type Rating of Ayrshire Females as Young Heifers and as Cows. GEORGE HYATT, JR., AND W. J. TYLER, *West Virginia University*.
- P13 The Effect of Certain Vitamins and Minerals on Blood Carotene Values of Dairy Animals. DWIGHT ESPE, *North Dakota Agricultural College*.
- P14 Effect of Certain Soybean Products on the Concentrations of Carotene and Vitamin A in the Milk and in the Blood Plasma of Dairy Cows. R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN, *Iowa State College*.
- P15 Further Studies on the Relationship between the Feeding of Soybeans and the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE, J. C. SHAW, AND B. C.

- HATZIOLOS, *University of Maryland*, AND L. A. MOORE AND J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*
- P16 The Influence of Tocopherols on the Fat Content of Milk. F. WHITING AND J. K. LOOSLI, *Cornell University*.
- P17 Covitamin Studies of Milk Fats from Four Breeds of Dairy Cattle. V. N. KRUKOVSKY AND F. WHITING, *Cornell University*.
- P18 Heat Production and Cardiorespiratory Activities during Gestation and Lactation in Jersey Cattle. S. BRODY, D. M. WORSTELL, H. H. KIBLER, AND A. C. RAGSDALE, *University of Missouri*.
- P19 A Biochemical and Histo-pathological Study of Ketosis in Dairy Cattle. J. C. SHAW, B. C. HATZIOLOS, AND V. P. SAARINEN, *University of Maryland*.
- P20 A Study of Sampling at Various Stages of Milking in Determining the Bacterial Flora of the Udders of Dairy Cows. E. M. KESLER, C. B. KNOTT, AND J. T. REID, *Pennsylvania State College*.
- P21 A Permanent and Convenient Rumen Fistula for Dairy Cows. G. E. STODDARD AND N. N. ALLEN, *University of Wisconsin*.
- P21-a Studies Bearing on the Bloat Problem. H. H. COLE AND MAX KLEISER, *University of California*.

4:30- 5:30 **COMMITTEE MEETINGS.**

Tuesday, June 15

Morning Session

- 9:00-12:00 **SECTION A, CALF PROBLEMS.** G. H. WISE, *Chairman*.
- P22 Calf Losses in a Self-contained Herd over a Period of 17 Years. R. E. JOHNSON, E. L. JUNGHERR, AND W. N. PLASTRIDGE, *University of Connecticut*.
- P23 The Effect of Prepartum Vitamin A Supplementation on the Newborn Calf. A. A. SPIELMAN, H. D. EATON, J. K. LOOSLI, AND K. L. TURK, *Cornell University*.
- P24 The Utilization of Fetal Liver Stores of Vitamin A by the Newborn Calf. A. A. SPIELMAN, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut*

- P25 Effect of the Method of Administration of Carotene and of Vitamin A upon the Rate at Which They Are Absorbed from the Alimentary Tract of Dairy Calves. N. L. JACOBSON, G. H. WISE, AND R. S. ALLEN, *Iowa State College*.
- P26 Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced in Calves by Ration Changes. W. C. JACOBSON AND J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*
- P27 Influence of the Ration on Some Blood Vitamin Constituents of the Young Dairy Calf. JOHN W. HIBBS AND W. D. POUNDEN, *Ohio Agricultural Experiment Station*.
- P28 Influence of the Ration on the Digestive Tract Microorganisms of the Young Dairy Calf. W. D. POUNDEN AND JOHN W. HIBBS, *Ohio Agricultural Experiment Station*.
- P29 Relation of Aerobic Bacterial Flora to Consistency of Feces. M. D. VAN PELT, R. E. JOHNSON, AND W. N. PLASTRIDGE, *University of Connecticut*.
- P30 Raising Dairy Calves without Colostrum. J. T. MILES, S. A. HINTON, AND HOMER PATRICK, *University of Tennessee*.
- P31 A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN, AND F. THORP, *Michigan State College*.
- P32 Effect of Tryptophan in the Diet on the Excretion of Niacin and Its Metabolic Products in Dairy Calves. G. C. ESH AND T. S. SUTTON, *Ohio State University*.
- P33 Performance of Calves on a Photolysed Milk Diet. R. G. WARNER AND T. S. SUTTON, *Ohio State University*.
- P34 Anemia in Young Calves and Its Alleviation by Iron. W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

PAUL H. PHILLIPS, *Discussion Leader*.

9:00-12:00 SECTION B, **ARTIFICIAL BREEDING**. L. A. MOORE, *Chairman*.

- P35 A Method of Evaluating Bull Semen. TOM LUDWICK, D. OLDS, AND M. CARPENTER, *University of Kentucky*.

- P36 Vital Staining of Bovine Spermatozoa with an Eosin-aniline Blue Staining Mixture. H. E. SHAFFER AND J. O. ALMQUIST, *Pennsylvania State College*.
- P37 Turbidometric Assay of Hyaluronidase in Bull Semen. JOHN P. MIXNER AND JAMES E. JOHNSTON, *New Jersey Agricultural Experiment Station*.
- P38 Hyaluronidase and Bull Semen. J. E. JOHNSON, E. J. STONE, AND J. P. MIXNER, *New Jersey Agricultural Experiment Station*.
- P39 Effect of Testis Biopsy on Semen Characteristics. J. F. SYKES, W. J. SWEETMAN, AND P. C. UNDERWOOD, *Bureau of Dairy Industry, U.S.D.A.*
- P40 Spermatozoa Behavior in Bovine Cervical Mucus at Varying Stages of Estrus. H. A. HERMAN AND OTIS H. HORTON, *University of Missouri*.
- P41 Varying the Proportion of Egg Yolk in Diluters for Bull Semen. ERIC W. SWANSON, *University of Tennessee*.
- P42 A Study of the Types of Bacteria in Bovine Semen and Their Effect upon Motility. J. E. EDMONDSON, K. L. TALLMAN, AND H. A. HERMAN, *University of Missouri*.
- P43 Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. J. O. ALMQUIST, *Pennsylvania State College*.
- P44 Breeding Results with Bovine Semen Treated with Varying Amounts of Thyroxine. A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska*.
- P45 Measuring Breeding Efficiency by Pregnancy Examinations and by Non-returns. G. R. BARRETT, L. E. CASIDA, AND C. A. LLOYD, *University of Wisconsin*.
- P46 Order Number of Insemination and Conception Rate. G. R. BARRETT, C. A. LLOYD, AND R. A. CARPENTER, *University of Wisconsin*.
- G. W. SALISBURY, *Discussion Leader*.

Tuesday, June 15

Afternoon Session

1:30- 4:30 **JOINT MEETING WITH EXTENSION SECTION.**
E. H. LOVELAND AND G. H. WISE, *Co-Chairmen*.
Symposium—Reproductive Problems in Dairy Cattle. L. A. MOORE, *Leader*.

1. Infectious Disease as a Cause of Infertility. D. E. BARTLETT, *Bureau of Animal Industry, U.S.D.A.*
2. Functional Causes of Infertility and Methods of Treatment.
 - a. Hormone Disturbances } S. A. ASDELL, *Cornell University.*
 - b. Nutrition Disturbances }
 - c. Inheritance. L. O. GILMORE, *University of Minnesota.*
3. Possible Modes of Approach to a Study of Infertility. J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*
4. Activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association. P. H. PHILLIPS, *University of Wisconsin.*

4:30- 5:30 COMMITTEE REPORTS.

Dairy Cattle Health Committee. L. A. MOORE, *Chairman.*

Dairy Cattle Breeding Committee. E. J. PERRY, *Chairman.*

Breeds Relations Committee. H. A. HERMAN, *Chairman.*

1. Program of Purebred Dairy Cattle Association. G. A. BOWLING, *Sec.-Treas.*

Wednesday, June 16

Morning Session

9:00-11:00 SECTION A, PARTURIENT CHANGES IN BLOOD AND IN MAMMARY SECRETIONS. G. H. WISE, *Chairman.*

- P47 The Effect of Udder Inflation of Cows with Parturient Paresis on Blood Calcium, Magnesium and Inorganic Phosphorus. VEARL R. SMITH AND R. P. NEIDERMEIER, *University of Wisconsin.*
- P48 A Study of Citric Acid Levels in the Blood and Urine of Cows at Time of Parturition. T. H. BLOSSER, VEARL R. SMITH, AND H. A. LARDY, *University of Wisconsin.*
- P49 The Effect of Prepartum Milking on Some Blood Constituents of the Cow. R. E. JOHNSON, H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut.*
- P50 A Study of Some Blood Constituents of Cows not Milked Following Parturition. R. P. NEIDERMEIER AND VEARL R. SMITH, *University of Wisconsin.*

- P51 The Effect of Preparturient Milking on the Composition of Colostrum. A. H. VAN LANDINGHAM, C. E. WEAKLEY, R. A. ACKERMAN, AND GEORGE HYATT, JR., *West Virginia University*.
- P52 The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut*.
- P53 The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, R. E. JOHNSON, AND R. J. SLATE, *University of Connecticut*.
- P54 The Effect of the Form of Vitamin A and of Tocopherol Supplements of the Ration on the Concentration of Vitamin A and Carotenoids of Colostrum and Early Milk. D. B. PARRISH, GEORGE H. WISE, AND J. S. HUGHES, *Kansas State College*.
- T. S. SUTTON, *Discussion Leader*.

9:00-11:00 SECTION B, **FORAGES, HAY.** L. A. MOORE, *Chairman*.

- P55 Comparison of Barn-cured and Field-cured Alfalfa Hay. GILBERT H. ROLLINS AND PAUL M. REAVES, *Virginia Polytechnic Institute*.
- P56 Studies on Mow Curing of Baled Hay. W. A. KING, J. W. WILBUR, S. M. HAUGE, AND A. W. COOPEL, *Purdue University*.
- P57 Stack Finishing of Baled Hay with and without Heat. K. A. KENDALL, W. B. NEVENS, AND J. H. RAMSER, *University of Illinois*.
- P58 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and a Poor Quality Field-cured Hay. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, C. G. MELIN, W. J. SWEETMAN, W. H. HOSTERMAN, AND H. M. TYSDAL, *Bureau of Dairy Industry; Bureau of Plant Industry, Soils and Agricultural Engineering; and Production and Marketing Administration*.
- P59 Vitamin D Content of Forages as Affected by Various Curing Procedures. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
- P60 Comparison of Early-cut and Late-cut Lespedeza Hay for Milk Production. C. E. WYLIE, J. A. EWING,

ERIC W. SWANSON, AND J. N. MADDUX, *University of Tennessee.*

P61 The Influence of Various Hays on the Production, Vitamin Content, and Flavor of Milk. J. K. LOOSLI, V. N. KRUKOVSKY, AND G. P. LOFGREEN, *Cornell University.*

P62 Comparison of Digestion Coefficients of Sun-cured and Mow-cured Hays from the Same Field. O. M. CAMBURN, *University of Vermont.*

11:00-12:00 **BUSINESS MEETING.**

Wednesday, June 16

Afternoon Session

1:30- 3:00 **SECTION A, FEEDING AND MANAGEMENT.** G. H. WISE, *Chairman.*

P63 Lactating Factors for Dairy Cows in Dried Grapefruit Peel. R. N. DAVIS AND A. R. KEMMERER, *University of Arizona.*

P65 The Growth of Dairy Heifers Reared on Maximum Roughage with Varying Amounts of Grain. O. T. STALLCUP, H. A. HERMAN, AND A. C. RAGSDALE, *University of Missouri.*

P66 Wintering Dairy Heifers on Legume Hay. S. A. HINTON, J. T. MILES, AND C. E. WYLIE, *University of Tennessee.*

P67 Observations on Calves Dehorned with Antimony Trichloride-salicylic Acid-collodion Preparation. G. E. STODDARD, *University of Wisconsin.*

P68 Comparison between Various Methods of Cooling Dairy Cows in Summer. D. M. SEATH AND G. D. MILLER, *Louisiana Agricultural Experiment Station.*

P69 Relationship of Management to the Let-down of Milk. C. E. KNOOP, *Ohio Agricultural Experiment Station.*

P70 The Effect of Time of Milking after Milk Excretion on Total Milk Production. G. M. WARD AND VEARL R. SMITH, *University of Wisconsin.*

1:30- 3:00 SECTION B, FORAGES, PASTURES. L. A. MOORE, *Chairman*.

P71 Silage or Winter Pasture for Dairy Cattle. C. E. WYLIE, S. A. HINTON, AND L. R. NEEL, *University of Tennessee*.

P72 Sweet Sudan as a Forage Crop for Dairy Cattle. K. A. KENDALL AND W. B. NEVENS, *University of Illinois*.

P73 Pastures in Relation to Dairy Development in the South. R. H. LUSH, *University of Tennessee*.

P74 Irrigated Pastures for Dairy Cows. JOHN EWING, NELSON MADDUX, C. E. WYLIE, AND R. H. LUSH, *University of Tennessee*.

P75 Increasing the Production of Permanent Pastures through Renovation. J. B. SHEPHERD, R. E. WAGNER, R. E. HODGSON, W. J. SWEETMAN, AND C. G. MELIN, *Bureau of Dairy Industry and Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S.D.A.*

P76 Effect of Intermittent and Limited Winter Grazing of Rye Pasture on the Carotene and Vitamin A Content of Cows' Milk. R. G. WASHBURN AND C. F. MONROE, *Ohio Agricultural Experiment Station*.

3:00- 5:00 GENERAL BUSINESS SESSION. Auditorium, Dawson Hall.

7:00 BARBECUE, Amphitheatre.

PROGRAM OF EXTENSION SECTION

Monday, June 14

Afternoon Session

1:30- 4:30 RECORDS AND INTERPRETATION. E. H. LOVELAND, *Chairman*.

Opening Business Session.

E1 Report of Dairy Records Committee. CHARLES GEARHART, *Pennsylvania State College*.

E2 Seven Years of Central Laboratory Testing. J. E. STOLLARD, *University of Wisconsin*.

Discussion.

Tuesday, June 15

Morning Session

9:00-12:00 **TEACHING METHODS AND EXHIBITS.**—G. HEEBINK, *Chairman.*

E3 Report of Committee on Teaching Methods. I. L. PARKIN, *Pennsylvania State College.*

E4 Interdepartmental Cooperation on Dairy Extension. EVERT WALLENFELDT, GEORGE WERNER, AND CARL NETZKE, *University of Wisconsin.*

Explanation and Discussion of Exhibits, Auditorium, Dawson Hall

Afternoon Session

1:30- 4:00 **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS.** E. H. LOVELAND AND G. H. WISE, *Co-Chairmen.*

(See Production Section Program)

Wednesday, June 16

Morning Session

9:00-11:00 **4-H CLUB AND TESTING RULES.** E. H. LOVELAND, *Chairman.*

E5 Systems Used in Obtaining 4-H Club Calves. RALPH PORTERFIELD, *University of Maryland.*

E6 National and Regional 4-H Dairy Contests. M. J. REGAN, *University of Missouri.*

E7 Adoption of Practices as the Result of 4-H Dairy Work. J. C. NAGEOTTE, *Pennsylvania State College.*

E8 Suggested Revision of D.H.I.A. Rules and Regulations. CHARLES GEARHART, *Pennsylvania State College.*

Discussion.

Afternoon Session

3:00- 5:00 **GENERAL BUSINESS SESSION, Auditorium, Dawson Hall.**

7:00 **BARBECUE, Amphitheatre.**

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THE SPECTROPHOTOMETRIC DETERMINATION OF THE COLOR OF MILK

VICTOR NELSON

Central Research Department, Food Machinery Corporation, San Jose, California

In the commercial manufacture of evaporated milk, considerable effort is given to the production of milk of uniform quality throughout the year. While ideas on quality vary, flavor, color and viscosity generally are regarded as the chief factors in quality rating.

In order to have a record of quality ratings, some method of measurement must be used which can be related to an accepted standard. Viscosity can be determined easily, but color and flavor have been difficult to rate, since no convenient or wholly satisfactory standards have been available. In most laboratories color and flavor remain a matter of the personal judgment of the inspector. However, color can be referred to known standards. The purpose of this paper is to report on the spectrophotometer as a means of evaluating the color of evaporated milk and related products.

METHODS AND APPARATUS

Some years ago Webb and Holm (4) and more recently Bell and Webb (1) measured the color produced in the processing of evaporated milk by means of the Munsell system of disc colorimetry. This system is relatively convenient, inexpensive and fairly accurate in its specifications of color. However, its lack of high sensitivity excludes it from the measurement of the minute changes in color which accompany variations in the heat processing of milk, especially those changes occurring at the lower temperatures, *e.g.*, at 220° F.

In recent years several spectrophotometers of relatively low cost have been introduced, and among these the Beckman provides a reflectance attachment for measuring the color of opaque solids. This attachment is so designed that it easily can be adapted for the measurement of opaque liquids such as milk.

Since no containers for liquids were included in the equipment, it was necessary to construct them in the laboratory. The containers were constructed from tin plate, were circular in shape, 0.5 inch in depth and 1.125

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inches in diameter. The reference standard ordinarily used is a magnesia block, but some difficulty was experienced in obtaining a block of uniformly high reflectance. Hence, for the data herein reported, the standard used was one of the sample cups filled with reagent grade magnesium carbonate. For simplicity, this standard was considered as having a reflectance of 100 per cent. A weighed amount of milk was used in order to insure a constant depth of milk in the cup. Since the surface of the reference magnesia standard and the surface of the liquid should be at the same level for accurate comparison, the surface of the standard was lowered to the level of the milk by the insertion of a metal plate with a 1-inch diameter circular opening and proper thickness between the top of the cup and the retaining plate. Measurements then were made as usual over the wave front of the instrument.

EXPERIMENTAL

The experimental part of this work consisted in comparing the reflectance of milk samples before and after processing with the reflectance of the

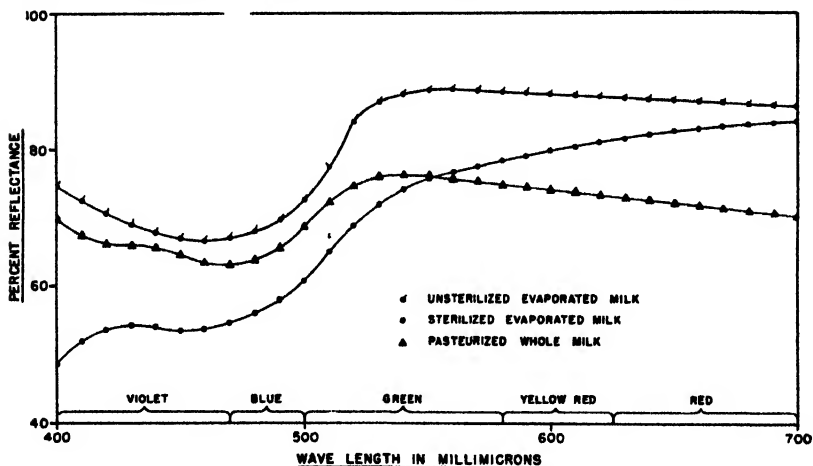


FIG. 1. Variations in reflectance values of pasteurized whole milk, sterilized milk and unsterilized milk between 400-700 mμ.

magnesia standard. These samples consisted of pasteurized milk, unsterilized evaporated milk, sterilized evaporated milk, and six lots of evaporated milk which had received varying preheater and sterilization treatments.

A comparison of the color of pasteurized milk with unsterilized evaporated milk and sterilized evaporated milk is shown by the curves on figure 1. The color difference between the three curves is indicated by their relative positions. The brightness of each color is indicated by the average level of the curve, *e.g.*, the color of unsterilized evaporated milk is the brightest.

with the pasteurized milk and the sterilized milk averaging about the same. An accurate statement regarding hue shift is possible only on the basis of colorimetric data to be calculated from these curves. The saturation of each color is indicated by the relative slope of the curves; *e.g.*, the pasteurized milk curve is the flattest, therefore the least saturated, while the other curves are steeper and therefore more saturated.

It should be noted that the spectrophotometer provides a means for the analysis of the spectral composition of a color sample, while the visual impression is the effect produced on the observer by the combined effect of the spectral composition of the sample, the spectral composition of the illuminant under which the sample is viewed, and the observer's own visual mechanism (which is more receptive to wave lengths in the middle portion of the visible spectrum than those on either end).

With regard to the data plotted on figure 1, it is interesting to note that concentration of milk produces an increase in reflectance in the green, yellow, and red wave lengths but little change in the blue and violet. There

TABLE 1
Conversion of curve data into I.C.I. and Munsell notation

Curve	I.C.I. color notation			Munsell color notation		
	x	y	Y	Hue	Value	Chroma
Pasteurized milk	0.3330	0.3470	0.738	7.3Y	8.8	1.7
Unster. evap. milk	0.3315	0.3435	0.853	5.8Y	9.3	1.55
Ster. evap. milk	0.3435	0.3500	0.738	1.7Y	8.8	2.3

is a possibility that the decrease in the blue-violet region is produced in the forewarming and evaporation processes.

Sterilization of milk produces a marked decrease in reflectance at all wave lengths, especially marked in the violet region. This inequality in reflectance loss is the primary reason for the brown appearance of sterilized evaporated milk, since the result is a relative increase in red and yellow and not an actual increase in these colors.

The conversion of the spectrophotometric data into the I.C.I. (2) and Munsell notation (3) is given in table 1. The Munsell values derived are in good agreement with those obtained by Bell and Webb (1) on evaporated milk. Therefore, it appears that no serious error is introduced by the fluorescence of riboflavin or other compounds.

In the routine grading of freshly sterilized evaporated milk, it is desirable to know the relative color of milk in terms of a simple index number. While this index number cannot represent accurately the true color, it can indicate the direction of shift in hue, brightness and chroma and thus afford to the inspector a quick estimate of the change in color. Since the

heat treatment of milk produces a loss of reflectance, especially marked in the green region, a wave length of 520 $m\mu$ appears particularly suitable for routine work on standard evaporated milk, since small visual changes give large instrumental readings. Furthermore, no special light bulb is needed, since the ordinary light source is relatively strong at this wave length.

The data plotted on figure 2 provide a comparison of the spectral composition of sterilized and unsterilized milk over a greater range than that provided by the data in figure 1. In this second experiment it was desirable to measure the degree of darkening, from a visual point of view, produced in the high temperature pretreatment of evaporated milk and in the subse-

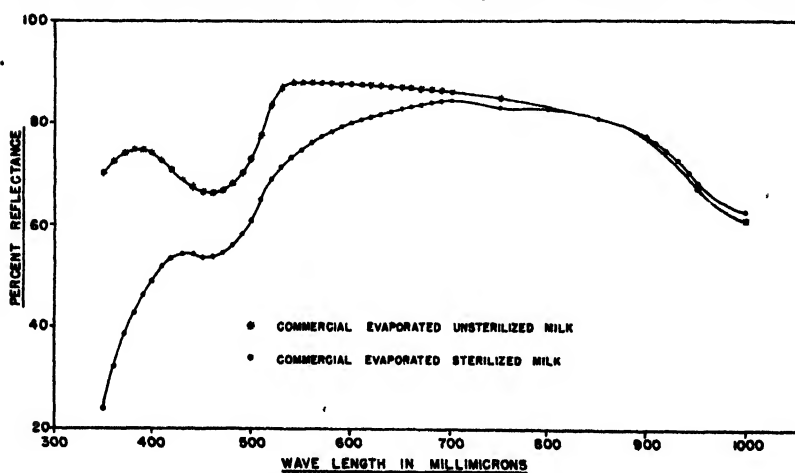


Fig. 2. Variations in reflectance values of sterilized and unsterilized milk between 350-1000 $m\mu$.

quent sterilization. Since these data are from pilot plant research on high temperature-short time sterilization, a note of explanation is offered.

The data are presented because they represent extreme time-temperature variations in treatment, interesting for the purpose of illustrating the effect on color values. A brief outline of the data follows: The treatment raised the temperature of the milk to the desired temperature in a few seconds. The pretreatment time (table 2) refers to the time the evaporated milk was held at the given temperature in a jacketed holder. The corresponding reflectance value was that taken after rapid cooling of the milk.

The cooled evaporated milk was filled into cans of 14.5-ounce capacity. The filled cans then were closed and treated in an experimental continuous sterilizer as follows: The cans were conveyed through a pre-heating chamber, the temperature of which increased at a uniform rate from 225° F. at the portal of entrance to 235° F. at the portal of exit of the cans. The cans of

TABLE 2
The effect of pretreatment and sterilization on the reflectance value of milk
(Sterilization time was 4.7 min.)

Group	Lot no.	Pretreatment temp. (°F.)	Pretreatment time (min.)	% Reflectance 520 mμ	Sterilizer temp. (°F.)	% Reflectance 520 mμ	Viscosity M.U.*
I	A	215	0	78.8	261	67.7	92
	B	"	8	78.3	260	68.2	50
	C	"	16	77.5	255	68.2	70
	D	"	24	75.9	254	66.2	85
	E	"	32	74.4	255	67.7	32
II	A	220	0	80.2	262	68.7	47
	B	"	5	80.1	256	66.5	48
	C	"	10	78.4	259	67.7	66
	D	"	15	77.4	255	64.5	80
	E	"	20	73.8	252	65.4	66
III	A	230	0	80.2	261	69.6	41
	B	"	4	79.3	259	66.2	25
	C	"	8	77.6	259	65.4	70
	D	"	12	75.7	254	62.2	125
	E	"	16	77.5	252	62.2	106
IV	A	240	0	76.9	260	68.0	39
	B	"	3	78.0	259	64.8	20
	C	"	6	73.9	259	61.6	68
	D	"	9	73.5	254	58.6	105
	E	"	12	71.2	252	62.2	52
V	A	250	0	77.6	262	69.0	19
	B	"	2	75.7	259	58.4	50
	C	"	3.5	75.4	257	59.8	50
	D	"	5	71.8	254	57.2	15
	E	"	6	71.2	262	60.7	37
VI	A	260	0	75.6	260	67.3	22
	B	"	0.5	74.4	262	58.4	18
	C	"	1.0	75.0	262	59.2	20
	D	"	1.5	66.0	254	51.3	12
	E	"	2.0	67.7		57.3	15

* M.U. = (Centipoise + 10)/1.9 (approximate).

milk then were conveyed through a second chamber, where they were subjected to the indicated temperature for 4.7 minutes. After this sterilization treatment the samples were cooled in the usual manner and tested for reflectance loss and viscosity. Viscosity values are in terms of Mojonnier units. There was no "burn-on" or other abnormality which would affect viscosity or color values.

Some discrepancies may be noted in the data. These could well be due to variations in the color of the original milk used and to some unavoidable departures from the temperatures given. In the case of the sterilized product, variations in the rate of cooling affected the color. In any case deviations from the expected color are not large when considered from the standpoint of visual perception, except for some notable exceptions in Groups V and VI. In these latter groups considerable unexplained variation was found in the sterilizing and color characteristics of the various lots of milk.

SUMMARY

1. The Beckman spectrophotometer provides basic data for spectral composition of energy reflected from a sample and when it is combined with standard colorimetric data (as the I.C.I. Standard Observer and one of the I.C.I. Standard Illuminants) it provides a good means of estimating the color.

2. A convenient index for routine estimations of the darkening in color of evaporated milk can be determined by noting changes in reflectance of light of 520 $m\mu$ wave length.

The author is indebted to Mr. Paul C. Wilbur, A. E. Peck and Dr. C. R. Stumbo for their valuable suggestions and criticism.

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THE COLOR OF EVAPORATED MILK WITH RESPECT TO TIME AND TEMPERATURE OF PROCESSING

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It is well known in the evaporated milk industry that the color of evaporated milk can be improved by using a high-temperature-short-time sterilization process. Recently, Tarassuk (3) showed that color in evaporated milk could be reduced a significant amount by reducing the oxygen content of the milk before sterilization. However, there is almost no information available, except that given by Bell and Webb (1), on the rates of color formation at the various sterilization temperatures.

Information on the rate of color development always has been desirable, but until recently no entirely satisfactory method has been available. In this paper the technique of color measurement used by Nelson (2) is applied to the investigation of the rates of color formation during the processing of evaporated milk.

METHOD AND APPARATUS

The apparatus consisted of a thermostatically controlled oil bath, a preliminary heating oil bath maintained at 175 F., 75 mm. \times 10 mm. test tubes, a wire tray for holding the tubes, a cold water bath for cooling the tubes quickly after heating and a Beckman spectrophotometer for reflectance measurements.

Because of the small milk sample used, a small container made from plastic was used instead of the larger container used by Nelson (2). Tests were made to insure comparableness of the two containers.

One and one-half milliliters of commercial unsterilized evaporated milk of 26 per cent total solids content was inserted carefully into the small tubes with the aid of a hypodermic needle. The tubes were sealed over a small pointed flame, the hot tip being drawn into a loop so that it could be suspended on a wire and placed in the wire basket.

The desired number of tubes filled with the evaporated milk was placed in the basket and held in the preliminary oil bath for 3 minutes before immersion in the constant temperature process bath. After immersion in the process bath, tubes were withdrawn at stated intervals, cooled in the water bath, dried, numbered and later analyzed.

EXPERIMENTAL

The data obtained in this work are represented graphically in figures 1 to 5.

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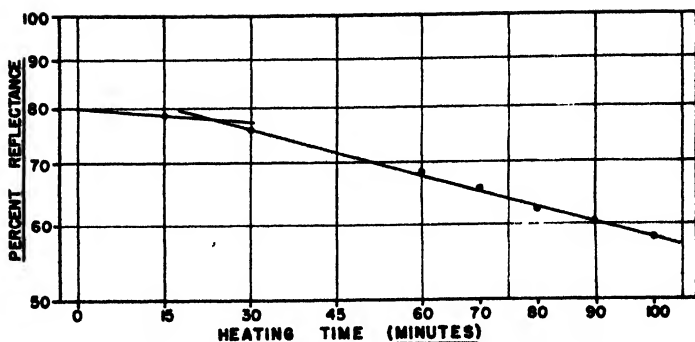


FIG. 1. The relationship between time of heating at 220° F. and reflectance at 520 $m\mu$ wave length.

The preliminary heating of the tubes to 175° F. is not essential but it is convenient, since the time then necessary to arrive within a degree of the desired temperature in the process oil bath is reduced to approximately 3 minutes, as determined by thermocouple measurements and the well known logarithmic nature of the heat penetration curve. Consequently, zero time in this experiment is 3 minutes after immersion in the process oil bath.

The data are plotted on semi-logarithmic paper, since it was found that a straight line was obtained if the logarithm of the reflectance was plotted against time.

Some reflectance loss is noted at zero time at 250° F. (fig. 4). However, all the data are plotted without correction, since the lag in the reflectance loss at the lower temperatures, or the rate of loss, is so low that a measurable reflectance loss cannot be found for several minutes. In any case, the error in zero time does not affect the slope of the curves, although it

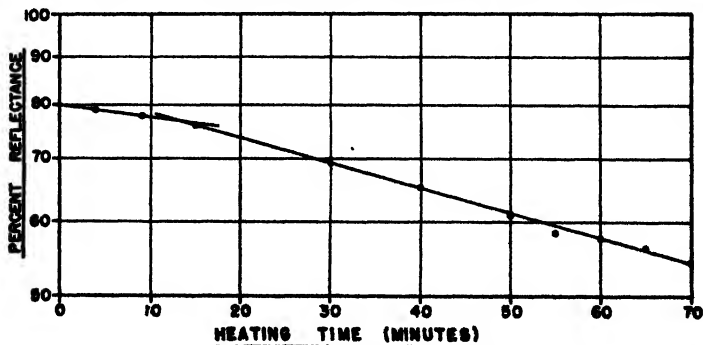


FIG. 2. The relationship between time of heating at 230° F. and reflectance at 520 $m\mu$ wave length.

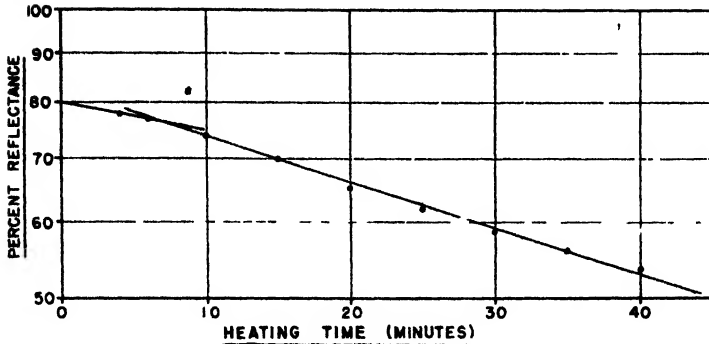


FIG. 3. The relationship between time of heating at 240° F. and reflectance at 520 $m\mu$ wave length.

does affect, to a minor degree, the values of the 250° F. curve. However, if it is desired to correct for this loss it seems not unreasonable to assume that projection of the curve until it crosses the 80 per cent line will give the time—about 80 seconds in this case—which should be added to the time plotted. (The reflectance of the original milk was 79.8 per cent at 520 $m\mu$ wave length.)

Plotting the data on semi-logarithmic paper was found advantageous. The data for the 250° F. curve are represented by a single straight line, while the data for the other curves are represented most conveniently by two straight lines. While the data for the short curves are inconclusive in determining the character of the curves, they are represented as straight lines for convenience and also to indicate the change in slope of the longer curves. In any event, there is a lag in the darkening in color of evaporated milk during processing, a situation also noted by Townley and Gould (4), who found that a visible color change occurred at the time of marked de-

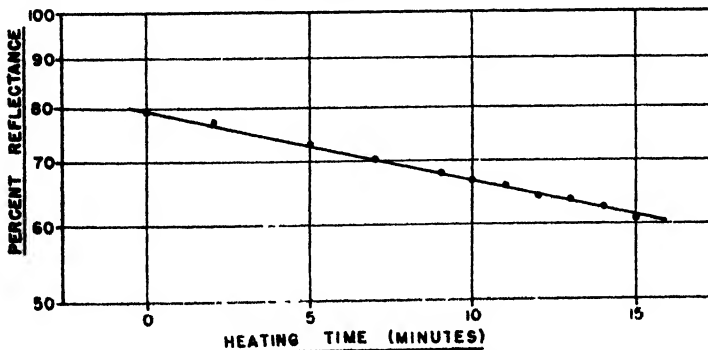


FIG. 4. The relationship between time of heating at 250° F. and reflectance at 520 $m\mu$ wave length.

crease in labile sulfur liberation. Whether this point of decrease marks a decided increase in the oxidation-reduction potential has not been determined, but in view of the effect of oxygen on color, it may be significant.

Curves 1 and 2 on figure 5 were derived from the slopes of the curves marked 220°, 230°, 240° and 250° F. The numerals on Curve 1 indicate the time in minutes at these particular points for which this curve is valid. After this time period, values should be selected from Curve 2.

In connection with this experiment, it should be noted that the ratio of volume of air to milk is greater than in commercial canning. Therefore,

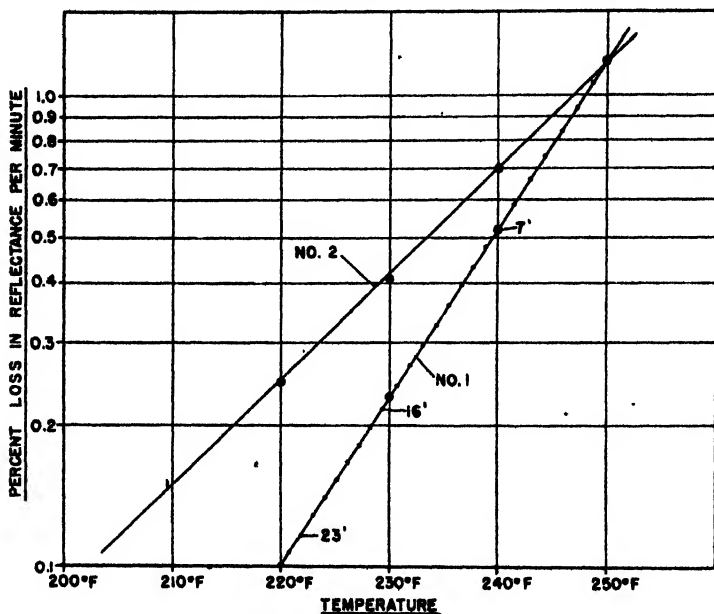


FIG. 5. The relationship between temperature and reflectance loss per minute.

it is quite possible that Curve 1 represents the condition of minimum lag period likely to be encountered in commercial practice. On the other hand, Curve 2 normally will represent the conditions prevailing after the lag phase of sterilization is concluded.

The data obtained in this experiment find application in the color evaluation of sterilization processes. While it is difficult to arrive at absolute values because of the variables introduced by the pretreatment a milk receives, the relative color values of processes can be determined with reasonable accuracy. Given the heat penetration curve of a process, a new curve can be constructed by substituting rate of reflectance loss values for temperature and integrating graphically the curve produced. For example, it will be found that the color produced in a commercial cooker

process of 15 minutes at 243° F. is greater than a comparable process of 6 minutes at 254° F. Not only is the high temperature process short, but the reflectance loss values are relatively low, since a large part of the process occurs in the lag phase of the curve.

SUMMARY AND CONCLUSIONS

1. The loss in reflectance at the temperatures studied decreased logarithmically with time after a lag period.
2. A lag period in reflectance loss was noted at temperatures below 250° F. The character of this curve is not known with certainty.
3. The data obtained are applicable to the color evaluation of sterilization processes.

The author is indebted to Mr. Paul C. Wilbur, A. E. Pech and Dr. C. R. Stumbo for their valuable suggestions and criticism.

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EFFECT OF RAW SOYBEANS AND OF SOYBEAN OIL ON PLASMA CAROTENE AND ON VITAMIN A AS MEASURED BY ACTIVATED GLYCEROL DICHLOROHYDRIN¹

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Interference with vitamin A metabolism in dairy cows fed soybeans and soybean products has been reported previously. Hauge *et al.* (3, 4) were among the first to demonstrate a factor in soybeans that suppressed the transfer of the vitamin A potency of the ration to the milk fat. These workers found that the factor could be removed from soybean oil by adsorption on activated charcoal. Although their data showed no lowering of carotene, they stated that carotene as well as the vitamin A values may be lowered by additions of large amounts of either soybeans or soybean oil to the ration. Cannon *et al.* (1) observed a bleaching effect on the milk fat of cows fed raw soybeans. Their observations were based on color comparisons rather than chemical determinations of vitamin A and carotene. Shaw *et al.* (6) recently reported the occurrence of a vitamin A deficiency in dairy calves from dams fed raw soybeans.

The effects of raw soybeans and soybean oil on the blood plasma carotene and vitamin A concentrations of lactating cows fed alfalfa hay, silage, concentrates and a carotene supplement are reported herein.

EXPERIMENTAL

Procedure. In this feeding trial either 9 lb. of raw soybeans or 1.7 lb. of expeller-process soybean oil, an amount calculated to be equivalent to the oil supplied by the raw beans, were incorporated into the rations of dairy cows to test their effect on the concentrations of blood plasma carotene and vitamin A. This quantity of raw soybeans, based on previous experiments (1), was selected as the probable maximum amount that could be fed daily over a prolonged experimental period.

Six Holstein cows were divided into two comparable groups. One of three experimental rations was assigned at random to each cow of Group I; these rations were duplicated for Group II. The daily feeding schedule indicating the concentrates fed is presented in table 1.

In addition to the concentrates, all cows were fed a poor quality alfalfa hay throughout the trial. Corn silage was provided for the first 6 weeks of the experimental period, at the end of which time the supply was exhausted and alfalfa hay became the only roughage. Before the trial started it was found that the cows selected had plasma carotene levels that were

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less than 300 γ per 100 ml. Since it was desired to conduct the studies with cows having more plasma carotene than this, so as to allow sufficient latitude for a possible depression, each cow also received daily both before and during the trial 0.5 lb. of a carotene preparation* containing 250,000 USP units of vitamin A per pound. This 0.5 lb. of carotene preparation was mixed with 2 lb. of the basal concentrate mixture and fed each cow

TABLE 1

Daily concentrate feeding schedule of the three cows in each of the two groups during each period

Cow no	Group no	Basal concentrate mixture* (lb)	Materials tested (lb)
Basal period (2 weeks)			
1947	I	14.0	
2470	I	13.0	
2379	I	15.5	
2210	II	14.0	
2392	II	15.0	
2472	II	14.0	
Experimental period (9 weeks)			
1947	I	5.0	9.0 Raw soybeans
2470	I	11.3	1.7 Soybean oil
2397	I	15.5	Control
2210	II	5.0	9.0 Raw soybeans
2392	II	13.3	1.7 Soybean oil
2472	II	14.0	Control
Cross-over period (4 weeks)			
1947	I	14.0	Control
2470	I	11.3	1.7 Soybean oil
2397	I	6.5	9.0 Raw soybeans
2210	II	14.0	Control
2392	II	13.3	1.7 Soybean oil
2472	II	5.0	9.0 Raw soybeans

* The basal concentrate mixture consisted of 250 lb. of ground yellow corn, 250 lb. crushed oats, 200 lb. linseed oil meal, 100 lb. wheat bran, 9 lb. common salt, and 16 lb. bone meal.

between the morning and evening feeding periods apart from the soybean products in order to avoid possible *in vitro* destruction of the carotene (2).

The trial was initiated with a 2-week basal period during which the plasma of each cow was characterized for its carotene and vitamin A content. An experimental period of 9 weeks followed the basal period. At the end of the 9-week experimental period the rations of the cows fed the control diet and those fed the raw soybeans were switched (table 1). This cross-over period was continued for 4 weeks. All other experimental

* "Super Carex", a carrot oil preparation taken up in a dry carrier, was obtained from Nutritional Research Associates, Inc., South Whitley, Indiana.

conditions were maintained with these two groups. The cows receiving the soybean oil, however, were continued on their starting experimental ration throughout the trial.

Venous blood samples were collected weekly from each cow and were analyzed immediately for vitamin A and carotene contents. Sufficient blood was drawn to supply duplicate 9-ml. plasma samples. Kimble's (5) procedure was used for extracting the vitamin A and carotenoids from the plasma. Five-milliliter portions of these extracts were used for determining the carotenoids. The per cent transmission readings obtained with a Coleman Universal Spectrophotometer set at 440 m μ were converted into carotene values by means of a standard curve.³ A new reagent, activated glycerol dichlorohydrin (G.D.H.), was used to determine the vitamin A of the blood plasmas. G.D.H. was selected in view of the potential advantages of this colorimetric reagent (7).

For the determination of vitamin A, 12-ml. portions of the plasma extracts were placed into 50-ml. centrifuge tubes. These tubes were heated in a water bath, which at no time exceeded 65° C., to evaporate the solvent. Immediately following the removal of the solvent, the tubes were cooled to room temperature and the residue in each tube dissolved in 1.5 ml. of chloroform. One milliliter of each chloroform solution was transferred to a Coleman cuvette and 4 ml. of G.D.H. added. The contents of the cuvette were mixed by inversion and the color allowed to develop for 4 minutes in the dark at room temperature, after which the readings were made with the spectrophotometer. The transmission readings were converted into vitamin A values by means of the standard curve and then corrected for carotene interference.

Validity of the reagent used for the determination of vitamin A. Sobel and Werbin (8) previously have shown G.D.H. to be satisfactory for the determination of the vitamin A of fish oils. Since information on the applicability of G.D.H. for the determination of vitamin A of bovine blood plasmas was unavailable, it was necessary to ascertain its validity for the type of study reported herein.

A series of recovery studies was made on pooled samples of bovine plasmas containing from 400 to 550 γ carotene per 100 ml. Natural vitamin A ester was added at four different levels and 95.9 to 100.0 per cent recovery was obtained using G.D.H. as the colorimetric reagent. These results indicate that vitamin A could be determined satisfactorily with G.D.H.

³ A Coleman Universal Spectrophotometer, Model 11, was used for all analyses. It previously was standardized at 440 m μ with crystalline B carotene for estimating the carotenoids, and at 550 m μ with a natural vitamin A ester, PC 3 capsule, obtained from Distillation Products, Inc., for estimating the vitamin A. A carotene interference curve was plotted from data obtained by the addition of G.D.H. to crystalline B carotene in chloroform.

TABLE 2
The effect of raw soybeans and soybean oil on plasma carotene and vitamin A

Weeks	Group I						Group II											
	Control			Raw soybeans			Soybean oil			Control			Raw soybeans			Soybean oil		
	Carotene		Vit. A	Carotene		Vit. A	Carotene		Vit. A	Carotene		Vit. A	Carotene		Vit. A	Carotene		Vit. A
	(γ/100 ml.)																	
Basal period																		
1																		
2	307	43.2	343	30.0	394	37.4	394	36.3	367	31.2	425	30.3	446	37.6				
			338	37.9	422				388	42.0	442	34.8	458	39.0				
Experimental period																		
1	365	47.3	324	45.5	422	34.9	430		430	48.0	420	40.9	418	47.0				
2	408	45.6	317	36.8	420	53.0	437		437	32.3	367	39.8	449	35.1				
3	550	33.4	398	43.7	518	30.0	499		499	37.2	386	45.8	480	30.4				
4	598	31.0	346	26.0	382	30.1	422		422	30.5	314	34.4	473	31.0				
5	504	39.2	350	40.0	396	33.2	422		422	33.0	259	42.6	514	32.5				
6	480	40.0	420	41.6	458	41.8	420		420	33.7	317	34.8	593	41.0				
7	662	34.6	420	24.4	509	26.5	535		535	30.9	341	33.6	533	29.2				
8	662	47.0	425	36.0	550	45.4	535		535	45.3	427	45.0	480	39.3				
9	571	37.0	437	40.3	566	41.2	564		564	45.4	403	39.5	490	40.6				
Cross over period ^a																		
1	427	38.1	502	26.3	599	24.7	482		482	27.3	461	34.7	456	22.1				
2	398	25.0	542	25.8	518	21.1	427		427	32.1	470	26.0	446	26.5				
3	432	27.8	553	39.4	542	36.6	432		432	40.2	490	44.7	394	31.0				
4	418	31.2	533	35.5	499	28.3	391		391	33.9	494	34.0	434	44.1				

^a The cows fed soybean oil were not switched but continued on the same ration in this period.

RESULTS AND DISCUSSION

The effects of feeding raw soybeans or soybean oil were measured by the changes that occurred in the concentrations of blood plasma carotene and vitamin A found in the cows fed these products. These changes also were compared with those that occurred in the blood plasma carotene and vitamin A of the cows fed the control ration.

In table 2 are listed the amounts of carotene and vitamin A that were found in the blood plasma of each of the cows at weekly intervals. In order to get a clearer picture of the changes that occurred in the concentration of carotene in the blood plasma of the cows under the three feeding

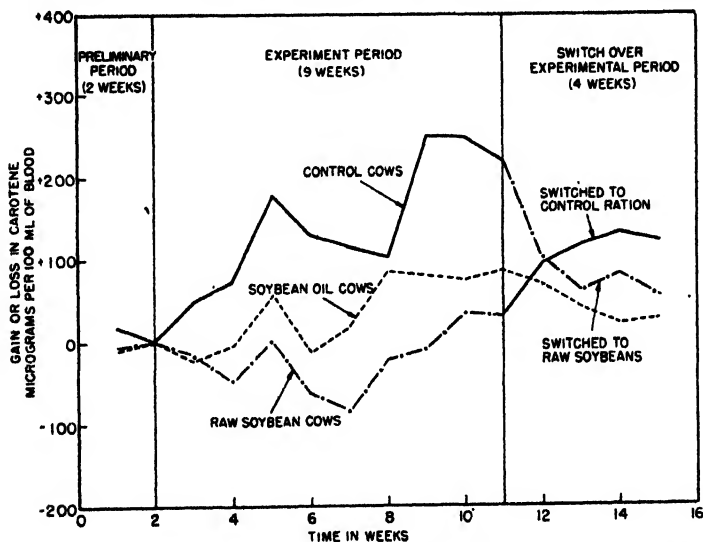


FIG. 1. Variations from the final determinations in a preliminary period, of average blood plasma carotene content for control cows, cows fed raw soybeans and cows fed soybean oil.

schedules, the data were plotted out as shown in figure 1. The zero point on these curves is the average concentration of carotene in the blood plasma at the end of the preliminary period for cows fed each feed. The changes in carotene concentration are plotted from that point.

It is apparent from the curves that the feeding of raw soybeans to cows caused their plasma carotene to decline somewhat in concentration and to remain considerably under that of cows fed the control ration. During the first 4 weeks the differences between these two lots of cows increased rapidly, then more slowly up to 7-9 weeks, when differences in concentration between the control cows and those fed raw soybeans seemed to level off at about 250 γ carotene per 100 ml. of blood plasma.

That this difference in carotene concentration was effected by feed and not by cow differences is strongly supported by what happened when the feed of these cows was switched. Those cows formerly being fed raw soybeans and then fed the control ration showed increasing concentrations of carotene from the time the rations were changed. In opposition, those cows which were changed from the control ration to raw soybeans showed a constantly decreasing carotene concentration in their blood plasma until it was considerably under that of the other group with which its ration was switched. Although the switch-over period lasted only 4 weeks, yet in this time the differences in plasma carotene concentration reached almost one-half the magnitude that existed in a similar period before the switch-over was made.

The changes in blood plasma carotene concentration of the cows receiving soybean oil were intermediate, lying between those of the control cows and those fed raw soybeans. Apparently the oil depressed the plasma carotene concentration but not to the extent of the raw beans. The effects of feeding the oil are not as clear as with feeding raw soybeans, since no switch-over of rations was made with these cows. It is not known whether their position in relation to the control cows would have been reversed had these rations been switched. Presumably such a result would have occurred.

As was noted in outlining the feeding procedure, changes in the kind of roughages that were fed occurred during the progress of the trial. At the end of the sixth week corn silage was eliminated from the ration and alfalfa hay fed in greater amounts. Also, during the fifth week (third week of the first experimental period) all the cows were inadvertently permitted access to fresh grass for approximately 2 hours.

These changes in feed no doubt affected the carotene intake of the cows. Since all cows were fed alike and supposedly increased their carotene intakes together, these changes should have caused no serious influence on the differences in carotene concentrations between groups. Increased or decreased intakes of carotene would cause fluctuations in the plasma carotene, but each group would be affected in the same way.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and the soybean oil rations (table 2) were small and showed no particular trends. If destruction of vitamin A was being caused by either the raw soybean or soybean oil, the physiological processes of the cows quickly replenished the supply in the blood from carotene or from liver storage. Perhaps if the carotene intake of the cows were low enough, the feeding of raw soybeans and maybe soybean oil would cause a decline in the vitamin A concentration in their blood. Hauge *et al.* (3) have specified a factor that adversely affects the vitamin A concentration in milk fat. This factor might be operative on blood plasma vitamin A.

SUMMARY

Feeding raw soybeans in the amount of 9 lb. daily to lactating cows caused marked differences in their blood plasma concentration of carotene from that of cows fed a control ration containing no soybean products. During the first 4 weeks the differences increased rapidly, but by 7-9 weeks they seemed to level off at about 250 γ carotene per 100 ml. of blood plasma. The reversal in blood plasma concentrations of carotene that took place after a switch-over of rations was made between the cows receiving the control and the raw soybean rations indicates that the causative factor was the feed rather than the individuality of the cows.

The feeding of expeller process soybean oil to lactating cows caused differences in their blood plasma concentrations that were intermediate with the concentrations found in the blood plasma of cows fed a control ration containing no soybeans or soybean products and cows fed raw soybeans. The oil apparently depressed the carotene concentrations but not to the extent of the raw soybeans.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and soybean oil rations were small and showed no particular trends.

Activated glycerol dichlorohydrin, a new reagent for the determination of vitamin A, proved to be satisfactory for this determination in bovine blood.

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SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. I. GROWTH, BLOOD COMPOSITION, AND COST¹

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In a study of the relative value of a simple and a complex concentrate mixture for young breeding bulls, the composition of the whole blood and of the plasma was investigated on the chance that the feeds might reflect different physiological effects upon these tissues. Quantitative studies of various blood constituents have proved invaluable in experimental, diagnostic and clinical work, despite the variability in "normals" observed in different individuals, sexes, species, physiological functions, regions, seasons and climates. Most studies of bovine blood composition have concerned the female rather than the male.

Table 1 summarizes the levels of several constituents of whole blood and plasma of bulls as found in previous studies.

Since the literature involving the relationships of whole blood and plasma constituents of cattle to diet, age, physiological functions and pathological conditions is too extensive to be considered here, recognition has been given only to those data which reflect the blood picture of healthy bulls and which are pertinent to the present study.

The purpose of this study was to ascertain the effects of a simple and a complex concentrate mixture upon growth as determined by wither height and heart girth measurements and upon the concentration of some of the constituents of blood. The cost of maintaining breeding bulls on these feeding regimes was examined.

EXPERIMENTAL PROCEDURE

Sixteen Holstein bulls of similar blood lines were obtained at birth and reared under the same management and feeding regime until the commencement of the experiment. From these animals the 12 bulls used in this investigation were selected at 18 months of age on the basis of uniformity of age, size, and blood and semen pictures. The 12 bulls composed two groups of six each. Group I received a simple concentrate mixture of

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TABLE 1
Summary of previous studies on levels of several constituents of whole blood and plasma in bulls

Investigator	No. of animals	Age	Whole blood			Blood plasma			
			Hb	R.B.C.C. ^a	R.B.C.V. ^b	Ca	Inorg. P	Total proteins	Ascorbic acid
Abderhalden (1)	1	24 mo.	(%) 10.64	(mill./mm. ³) 8.24	(%) 33.43	(mg.%) 7.93	(mg.%) 2.73	(%) 6.97	(mg.%)
Kusner (12)	6	2 days-37 wk.	11.74	9.78
Knoop <i>et al.</i> (11)	5	1-5 mo.	10.52
Anderson <i>et al.</i> (2)	5	birth to 10 mo.	11.81
McClay (15)	6	Mature	12.80	12.62	3.34
Brook and Hughes (5)	24	..	11.92
Dimock and Thompson (7)	3	..	10.66	5.81
Lamarre (13)	4	6.50-10.90
Payne <i>et al.</i> (18)	45 27	12 mo. Over 12 mo.	10.46 13.03	7.30 4.76
Schwob (24)	10	7.28	..
Hortree <i>et al.</i> (3)	1	26 mo.	12.79	11.40	7.2	..	0.19-0.39
Hamerma (9)	1	27 mo.	12.18	10.60	7.5
Phillips <i>et al.</i> (19)	22	0.27

^a Red blood cell count.

^b Red blood cell volume.

which corn and corn gluten meal constituted a large portion, while Group II was fed a complex concentrate mixture (table 2). Both groups received the same average grade timothy-clover hay. The average composition of the concentrate mixtures and hay used during the feeding trial is shown in table 3.

The bulls were fed 1 lb. of hay per 100 lb. body weight daily with concentrate feed in sufficient quantity to provide an average daily digestible nutrient intake of approximately 1.02 and 1.18 lb. per 100 lb. body weight before and after an average age of 760 days, respectively. Feed intake was adjusted at average intervals of 43 days following heart girth and

TABLE 2
Composition of concentrate mixtures

Ingredients	Group I	Group II
	(%)	(%)
Ground yellow corn	54.0	10.0
Beet pulp	25.0	10.0
Corn gluten meal	10.0	10.0
Cane molasses	10.0	10.0
Linseed meal	10.0	12.0
Soybean meal	10.0	17.0
Crushed oats	10.0	25.0
Wheat bran	10.0	10.0
Dehydrated alfalfa	10.0	10.0
Limestone	10.0	2.0
Iodized salt	1.0	1.0
Bone meal	10.0	0.7
Brewers yeast	10.0	1.95
Mineral salt mixture ^a	10.0	0.1
Fish liver oil ^b	10.0	0.2
Irradiated yeast ^c	10.0	0.05
	100.00	100.00

^a Mineral salt mixture consisted of: $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 50%; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 44.5%; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5.0%; and $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 0.5%.

^b Fish liver oil containing 15,000 I.U. or more of vitamin A per g.

^c Irradiated yeast containing 9,000 USP units of vitamin D per g.

with height measurements. The average daily digestible nutrient intake immediately following body measurement was 1.08 and 1.22 lb. per 100 lb. body weight before and after an average age of 760 days, respectively, and regressed to about 0.96 and 1.11 lb., respectively, before the next adjustment as calculated from Morrison's tables (17). Body weight was calculated from the heart girth measurement according to the equation suggested by Branton and Salisbury (4).

An attempt was made to obtain about the same amount of semen from the bulls of each group; however, more was being taken from Group II during the latter part of the experiment than from Group I. The average daily semen volume and accumulative semen volume taken from the two groups are represented by the graphs and curves, respectively, shown in

TABLE 3
Average chemical composition of feeds (per cent of dry matter)

Group	Protein	Fat	Fiber	Ash	N.F.E.	P	Ca	Mn
Concentrate mixtures								
I	12.00	2.72	8.70	4.73	64.47	0.30	0.33	0.0044
II	21.76	4.67	10.08	9.03	49.03	0.66	0.97	0.0233
Hay								
	6.44	2.24	38.73	4.85	44.02	0.15	0.30	0.0053

figure 1, heart girth measurements also being included. The difference in the accumulative semen volume does not indicate that the bulls of Group II were capable of producing greater volumes of semen but merely that more semen was taken from these animals than from the bulls of Group I.

No data were obtained on the semen of one bull in Group II, since this animal manifested a fear which precluded obtaining semen from him in the usual manner. One bull was eliminated from Group I about mid-trial because of tuberculosis reaction. The animals were 18 months old at the beginning and 33 months old at the termination of the experiment. Twenty days was the greatest difference between the ages of any of the bulls.

The data on the phase of the study dealing with the blood constituents have been grouped into 3-month age periods for convenience of study. The

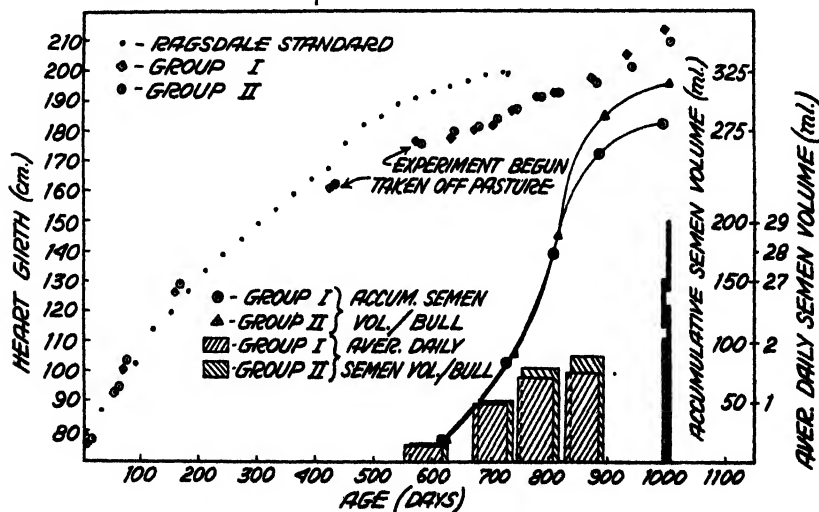


FIG. 1. Heart girth measurements of bulls, the Ragsdale standard (20) for Holstein bulls, and the average daily and accumulative volumes of semen produced per bull.

chemical methods used to determine the levels of certain blood and plasma constituents are as follows: hemoglobin, Sanford *et al.* (23); glutathione, Woodward and Fry (26); calcium, Clark and Collip (6); inorganic phosphorus, Fiske and Subbarow (8); phosphatase, method of King and Armstrong (10) as modified by Wiese *et al.* (25); plasma proteins, albumin and globulins, Looney and Walsh (14); and ascorbic acid, modification of method of Mindlin and Butler (16). The red blood cell count and volume (hematocrit) were determined on the same blood samples according to the standard procedures.

RESULTS

Growth. Good growth of bulls was effected by both feeding regimes once the retarded growth which was incurred on the late fall pasture

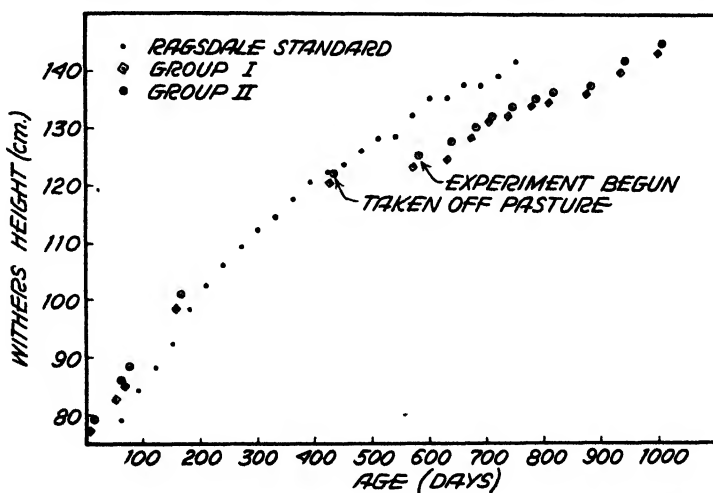


Fig. 2. Withers height measurements and the Ragsdale standard (20) for Holstein bulls.

prior to the commencement of the feeding trials was overcome (figs. 1 and 2). Semen production did not appear to affect growth (fig. 1). A study of the body measurements of the bulls of both groups would indicate a slightly more rapid growth of these animals during the actual feeding trial than is considered standard for Holstein bulls of the same age (20). It should be pointed out, however, that the standard proposed by Ragsdale (20) is based upon the measurements of only two bulls subsequent to 540 days of age. Group I animals gained body weight at an average rate of 0.18 lb. per day faster than Group II bulls. The rate of increase in height at the withers was similar for both groups (Group I, 1.57 and Group II, 1.54 mm. per day), as shown in figure 2. A similar general appearance and degree of fleshiness was observed in the animals of both groups.

TABLE 4
Blood constituents at various ages

Constituent	Groups ^a	Age (mo.)											
		18-21		21-24		24-27		27-30		30-33			
		No. cases ^b	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content		
Hb (gm. %)	I	12	12.07 ± 0.31	18	12.70 ± 0.29	23	13.50 ± 0.25	10	14.16 ± 0.31	15	14.49 ± 0.21		
	II	12	12.32 ± 0.26	18	13.15 ± 0.16	24	13.40 ± 0.13	12	14.09 ± 0.13	18	15.16 ± 0.16		
R.R.C.O. ^c (mill./mm. ³)	I	12	9.88 ± 0.38	18	9.56 ± 0.35	23	8.88 ± 0.25	10	8.85 ± 0.47	15	8.83 ± 0.31		
	II	12	9.98 ± 0.36	18	9.62 ± 0.24	24	8.62 ± 0.18	12	8.55 ± 0.25	18	9.36 ± 0.22		
R.R.C.V. ^c (%)	I	12	35.98 ± 1.60	18	33.59 ± 0.88	23	36.67 ± 0.78	10	37.74 ± 1.03	15	40.12 ± 0.77		
	II	12	35.76 ± 1.17	18	34.45 ± 0.52	24	35.58 ± 0.49	12	37.54 ± 0.86	18	40.26 ± 0.78		
Mean corpuscular	I	12	12.22	18	13.28	23	15.20	10	16.00	15	16.41		
Hb (γ)	II	12	12.34	18	13.67	24	15.55	12	16.48	18	16.20		
Mean corpuscular	I	12	36.42	18	35.14	23	41.30	10	42.64	15	43.01		
volume (μ ³)	II	12	35.83	18	35.81	24	41.28	12	43.91	18	43.01		
Reduced	I	12	28.66 ± 0.66	17	30.17 ± 1.20	23	34.79 ± 1.05	10	35.51 ± 2.12	15	41.12 ± 1.47		
glutathione (mg. %)	II	12	27.47 ± 1.38	18	31.22 ± 1.35	24	35.06 ± 1.02	12	34.40 ± 1.45	18	41.86 ± 1.21		
Oxidised	I	12	4.94 ± 0.53	17	8.84 ± 0.47	10	5.60 ± 0.83		
glutathione (mg. %)	II	12	6.54 ± 0.57	18	7.99 ± 0.49	12	6.95 ± 0.43		
Total	I	12	33.60 ± 0.71	17	39.01 ± 1.18	10	45.27 ± 2.13		
glutathione (mg. %)	II	12	34.01 ± 1.45	18	39.21 ± 1.24	12	48.44 ± 1.64		

^a Group I received simple concentrate mixture and mixed hay.
^b Group II received complex concentrate mixture and mixed hay.
^c Red blood cell count.
^d Red blood cell volume.

TABLE 5
Plasma constituents at various ages (mean and standard error)

Constituent	Group*	Age (mo.)									
		18-21		21-24		24-27		27-30		30-33	
		No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content
Ca (mg.%)	I	12	8.79 ± 0.22	18	10.49 ± 0.15	23	10.60 ± 0.10	10	11.02 ± 0.16	10	11.65 ± 0.38
	II	12	9.37 ± 0.22	18	10.09 ± 0.11	24	10.37 ± 0.09	12	10.61 ± 0.09	12	11.52 ± 0.74
Inorg. P (mg.%)	I	12	8.26 ± 0.18	18	7.76 ± 0.26	23	7.61 ± 0.21	10	7.13 ± 0.14	15	7.16 ± 0.24
	II	12	7.56 ± 0.24	18	7.78 ± 0.13	24	7.23 ± 0.26	12	6.86 ± 0.26	18	7.34 ± 0.18
Phosphatase, acid (units/100 ml.)	I							5	2.32 ± 0.18	10	1.43 ± 0.29
	II							6	1.72 ± 0.20	12	1.36 ± 0.19
Phosphatase, alkaline (units/100 ml.)	I					5	8.06 ± 0.39	10	6.09 ± 0.59	10	11.18 ± 0.56
	II					6	9.53 ± 1.81	12	7.61 ± 1.67	12	12.15 ± 0.88
Total proteins (g.%)	I	6	7.55 ± 0.06	18	6.81 ± 0.10	23	7.35 ± 0.07	10	6.80 ± 0.13	10	6.90 ± 0.10
	II	6	7.68 ± 0.17	18	6.99 ± 0.07	24	7.21 ± 0.08	12	6.90 ± 0.10	12	6.90 ± 0.07
Albumin (g.%)	I					5	2.91 ± 0.13	5	5.21 ± 0.08	9	4.05 ± 0.14
	II					6	4.14 ± 0.36	6	5.18 ± 0.13	12	4.02 ± 0.16
Globulins (g.%)	I					6	4.76 ± 0.25	5	1.82 ± 0.12	14	2.65 ± 0.14
	II					6	3.31 ± 0.35	6	1.93 ± 0.14	18	2.71 ± 0.11
Ascorbic acid (mg.%)	I	12	0.20 ± 0.01	18	0.28 ± 0.03	23	0.32 ± 0.02	10	0.37 ± 0.02	15	0.27 ± 0.02
	II	12	0.26 ± 0.02	18	0.27 ± 0.02	24	0.29 ± 0.01	12	0.34 ± 0.03	18	0.34 ± 0.01

* Group I received simple concentrate mixture and mixed hay.
Group II received complex concentrate mixture and mixed hay.

Blood composition. Remarkably similar values were found for various constituents and characteristics of the blood and plasma of the animals of both groups during the same age period. These data are summarized by groups in tables 4 and 5. Since no appreciable group differences were observed, the average data from both groups would seem to be normal for bulls of similar age. Although diet did not appear to influence the composition of the blood of these animals, various trends were observed which appeared to be associated with aging.

The concentration of hemoglobin, the red blood cell volume, and the mean corpuscular hemoglobin and volume gradually increased, whereas the number of erythrocytes decreased very little as age progressed from 18 to 33 months. Other constituents tending to increase with age were plasma calcium and reduced and total glutathione. The plasma level of inorganic phosphorus tended to decrease, whereas no definite relationship between the plasma concentration of ascorbic acid and age was observed.

The variations in alkaline plasma phosphatase were attributed to the rate of semen collection as reported previously (22). Likewise, the fluctuation in the levels of albumin and globulin may have been related to the production of semen.

Data on total and oxidized glutathione are not given for the periods 24 to 27 months and 27 to 30 months because estimations of this compound could not be obtained. Inability to measure this compound was concomitant with an increased rate of semen collection and appeared to be caused by a factor(s) existing in blood plasma under these conditions which prevented the reduction of oxidized glutathione by metallic zinc (21).

Cost. A comparable gain in body weight cost approximately 50 per cent more in Group II than in Group I. The average cost of maintaining a bull on the Group II regime was \$52.75 more per year than that of a bull receiving the other diet.

DISCUSSION

No important differences were found in the growth, general health, and blood constituents of two groups of breeding bulls receiving markedly different concentrate feeds. On the basis of these criteria, it would seem that costly, complex concentrate mixtures are not necessary for animals of similar age and producing semen at similar rates. The final evaluation of complex feeds, however, necessarily lies in their effects upon the production of fertile semen. A subsequent report in this series will consider the production of semen by the bulls used in this study.

Although this investigation revealed no group differences, various trends appeared to be associated with aging. In view of the lack of dietary influence, the concentration of some blood constituents investigated in this study would appear to be standard for bulls of similar age and breed when maintained under climatic conditions similar to those of northern New Jersey (tables 4 and 5).

The results of this study would seem to indicate the importance of the rumen in the nutrition of the bull. Regardless of the supposed limitations of the simple concentrate feed received by Group I animals, these bulls were able to maintain, at levels similar to those of bulls receiving a more complex diet, not only growth but also blood constituents believed to be indicative of physical well being. It should be pointed out that these relationships may not necessarily hold for bulls of greater age or for the same bulls over a longer period of time, as these data were obtained from bulls during the interval of 18 to 33 months of age. The importance of the poor to average grade hay fed to both groups may be underestimated in these considerations. Since hay and concentrates were fed in a manner believed to be consistent with good feeding practice, and since the same hay was fed to both groups, the main considerations involved comparisons of the effects of the two concentrate mixtures. Additional data dealing with the merits of these diets are presented in the subsequent paper on semen production of young bulls.

Although the cost of maintaining a sire by the ordinary breeder is of no great significance if satisfactory performance is being obtained, large bull studs such as those used in some artificial breeding units would effect a considerable saving by using simple concentrate mixtures similar to the one employed in this study rather than complex, high protein mixtures.

SUMMARY

1. Comparable rates of growth and concentrations of several blood constituents were found in bulls receiving a simple and a complex concentrate mixture.

2. Since the levels of certain blood and plasma constituents were similar for the two groups, these figures are presented as standards for healthy Holstein bulls of similar age and producing semen at similar rates.

3. The hematocrit, mean corpuscular hemoglobin and volume, the level of hemoglobin, reduced and total glutathione, and plasma calcium tended to increase with aging, whereas the plasma concentration of inorganic phosphorus decreased.

4. The maintenance of bulls on the complex concentrate feed cost approximately 50 per cent more than that of bulls receiving the simple concentrate mixture.

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SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. II. SEMEN PRODUCTION¹

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The comparative merits of simple and complex concentrate mixtures for semen production by bulls have not been ascertained previously. Various investigations, however, have demonstrated the efficacy and the lack of effect of certain feeds and specific nutritional factors upon the quantity and quality of semen ejaculated by bulls. Jones *et al.* (12) showed that rations which are satisfactory for normal growth to 3 years of age are adequate for normal reproductive performance. Recent Cornell investigations (6, 26) in which total digestible nutrient levels of 100, 120, and 140 per cent of the Morrison dry cow maintenance requirements (21) were fed to breeding bulls demonstrated that neither the quantity and quality of semen produced nor the fertility of the bulls was related to the digestible nutrient intake within the limits studied. Concentrate mixtures containing 12, 16, and 20 per cent total protein did not affect significantly the fertility of bulls (6, 26). However, bulls receiving the 20 per cent protein concentrate produced significantly greater concentrations of spermatozoa and lower ejaculate volume and motility and less total spermatozoa per ejaculate than did bulls receiving the other concentrate mixtures. For bulls in active service, these workers (6, 26) suggested feeding at the rate of 1 lb. of hay and 0.4 to 0.5 lb. concentrate mixture containing 12 per cent protein per 100 lb. body weight daily.

Jones *et al.* (11) found that bulls fed alfalfa hay supplemented with 1 lb. each of skim milk powder and oats groats daily grew faster, matured earlier, were in better condition, and produced good quality semen earlier than bulls receiving a basal ration of hay supplemented with salt, phosphorus and iodine. These differences were attributed to the greater energy intake rather than to the quality or quantity of protein ingested.

Since it was not possible in this experiment to use the semen from the unregistered bulls for breeding purposes, a number of measures of semen quantity and quality were employed as criteria of the relative merits of the two concentrate mixtures fed. Numerous reports support the use of the following tests of semen quality and quantity as an evaluation of rela-

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tive fertility: spermatozoa concentration (15, 16, 23, 29, 32, 34); initial motility (9, 15, 16, 20, 28, 30, 31); livability or maintenance of motility (1, 10, 17, 20, 30, 31, 34); pH (2, 3, 4, 8, 9, 10); change in pH upon incubation (1, 4, 16); number of morphologically abnormal spermatozoa (1, 10, 15, 19, 32, 36); semen and spermatozoa volume (1, 3, 7, 16, 32); semen level of ascorbic acid (8, 13, 14, 22); and reducing capacity (5, 27, 28, 33).

It was the purpose of this study to evaluate the relative merits of a simple and a complex concentrate mixture for the production of semen by young bulls as determined by various tests for semen quantity and quality.

EXPERIMENTAL PROCEDURE

A study of the semen obtained from the two groups of bulls (one receiving a simple concentrate mixture and the other a complex mixture) was made simultaneously with the investigation of the blood composition, growth, and cost of maintenance of these animals reported in the first paper of this series (25). For details on the composition of feeds, rate of feeding, age and growth of the bulls, and the cost of the concentrate feeds, the reader is referred to the previous paper (p. 429). Of the 12 animals composing the two groups (reported in the previous paper), five in each group yielded semen during the entire experiment.

Semen quantity and quality were studied during four periods ranging from 56 to 65 days in length, separated by rest periods of 14 to 51 days, as shown in table 1. These collection periods loosely represent the four seasons.

The quantity of semen produced was expressed as the number of ejaculates obtained, the average ejaculate volume, and the average daily semen volume per bull. The quantity of spermatozoa was determined by direct counts using a cytometer and by centrifugation of semen in hematocrit tubes. These data were expressed as the concentration of spermatozoa, the proportion of the whole semen volume consisting of spermatozoa, and the average size of a spermatozoan in terms of volume (cubic micra).

Since a constant temperature stage incubator was found to be necessary but was not obtainable during the first period, no attempt was made to procure data on motility and allied characteristics for this period. During subsequent periods, the initial motility, the motility at intervals following the initial estimation, and the livability of spermatozoa were determined by means of a constant temperature stage incubator adjusted to a temperature of 100° F. The data on motility are expressed in terms of arbitrary units derived from separate estimations of the number of living spermatozoa and the spermatozoa engaged in progressive motility. The motility units used here would be approximately equivalent to motility data expressed in terms of per cent ÷ 5. Livability of spermatozoa was calculated as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

TABLE 1
Semen characteristics and constituents

Period no.		I	II	III	IV	Summary ^b
Period date		3/5-5/9/46	6/29-8/28/46	9/12-11/14/46	12/5/46-1/30/47	3/5/46-5/22/47
Av. beginning and terminal ages of bulls	I ^a	556-621	672-732	747-810	831-887	556-998
	II ^a	564-629	680-740	755-818	839-895	564-1006
Volume	Av. ejaculate volume (ml.)	2.95(40) ^c 3.18(33)	3.52(110) 3.79(91)	3.70(144) 4.15(120)	4.13(101) 4.44(111)	3.82(423) 4.19(383)
	Av. daily semen volume/bull (ml.)	0.30	1.07	1.41	1.49	
		0.32	1.15	1.58	1.76	
Spermatozoa	Av. spermatozoa conc. (mill./mm. ³)	0.735(25) 0.775(23)	0.891(41) 0.962(34)	1.026(52) 1.020(45)	0.823(47) 1.087(47)	0.869(193) 0.950(177)
	Av. % spermatozoa ^d		6.62(31) 6.54(24)	7.44(51) 7.48(45)	9.24(42) 9.56(42)	7.84(124) 8.06(111)
	Av. spermatozoan volume (μ ³)		84.03(30) 72.86(24)	78.22(51) 77.63(45)	105.26(42) 90.63(42)	88.87(123) 81.52(111)
Motility	Initial motility ^e					
			13.31(36) 12.76(29)	13.17(52) 13.29(45)	11.05(47) 12.72(47)	11.98(163) 12.61(149)
	Motility at 100 hours ^f		2.55(34) 2.49(27)	5.65(52) 4.51(45)	7.54(47) 7.77(46)	5.53(133) 5.32(118)
Livability at 100 hours ^g			17.23(34) 19.68(27)	35.46(52) 31.82(45)	58.49(47) 52.93(46)	38.94(133) 37.27(118)
Reducing substances	Total reducing substances (mg.%)	30.32(19) 33.55(17)	37.30(41) 37.94(34)	36.48(47) 41.15(41)	26.47(21) 34.79(25)	34.18(128) 37.75(117)
	Reducing substances in oxidized state (mg.%)	15.89(13) 18.40(10)	14.07(41) 13.55(33)	13.55(38) 15.13(33)	5.84(21) 6.83(23)	12.58(113) 13.01(99)
	Potential reducing capacity (mg.%)	43.21(15) 53.59(12)	51.37(41) 50.73(33)	50.04(38) 56.05(33)	32.32(21) 39.26(23)	46.39(115) 50.19(101)
	Ascorbic acid (mg.%)	7.01(23) 8.59(22)	8.22(41) 7.96(34)	8.28(47) 9.32(40)	5.17(19) 7.08(23)	7.58(130) 8.36(119)
pH	Initial pH	6.86(16) 6.77(14)	6.55(34) 6.57(28)	6.68(50) 6.59(45)	6.70(25) 6.61(25)	6.67(138) 6.62(123)
	Post incubation pH	6.56(16) 6.56(14)	6.13(34) 6.19(28)	6.28(50) 6.33(45)	6.45(25) 6.41(25)	6.32(138) 6.35(123)

^a Roman numerals represent group number.

^b Summary includes data obtained during entire feeding experiment. (Therefore, data on semen obtained in a special study made of semen phosphatases subsequent to Period IV are included.)

^c Figures in parentheses indicate number of samples studied.

^d Represents proportion of total semen volume consisting of spermatozoa.

^e Estimated at 100° F. Motility value × 5 is approximately equivalent to per cent motility.

^f Motility rating at 100 hours subsequent to ejaculation (rating × 5 is approximately equivalent to the motility expressed as per cent).

^g Livability is expressed as the per cent of original motility persisting at 100 hours subsequent to ejaculation.

The reducing substances in semen were measured by a procedure similar to that outlined by Woodward and Fry (37) for the estimation of glutathione in whole blood (24).

Ascorbic acid was determined according to the method outlined by Mindlin and Butler (18).

The pH of semen was measured immediately after ejaculation and after incubation at 37° C. for 1 hour, using a Beckman pH meter equipped with a glass electrode. The decrease in pH effected by incubation was calculated from these estimations.

TABLE 2
Percentages of various types of morphologically abnormal spermatozoa

Abnormality	Group	Period				Av. over 426 days
		I	II	III	IV	
		(%)	(%)	(%)	(%)	(%)
<i>Head</i>	Pyriform	I 5.92	4.70	4.16	4.99	4.76
		II 4.49	2.31	2.05	1.94	2.48
	Tapering	I 2.60	1.04	1.04	0.99	1.28
		II 2.51	0.94	0.75	0.58	1.04
	Others	I 0.52	0.79	0.84	1.16	0.84
		II 0.34	0.45	0.76	0.63	0.59
<i>Midpiece</i>	Filiform	I 0.31	0.46	0.44	0.22	0.37
		II 0.49	0.48	0.32	0.23	0.36
	Beaded	I 0.54	1.15	1.59	1.46	1.28
		II 1.93	1.15	1.82	1.25	1.55
	Others	I 1.17	2.07	5.03	8.41	4.44
		II 0.57	1.60	4.25	9.64	4.34
<i>Tail</i>	Coiled	I 0.96	0.46	0.83	2.86	1.21
		II 1.26	0.94	0.63	1.60	1.03
	Beaded	I 0.32	0.12	0.35	0.34	0.29
		II 0.45	0.12	0.47	0.44	0.38
	Others	I 0.33	0.18	0.59	0.70	0.47
		II 0.56	0.09	0.72	0.62	0.53
Total abnormalities	I	12.67(22)*	10.97(33)	14.87(52)	21.13(30)	14.94(137)
	II	12.60(20)	8.08(27)	11.77(46)	16.93(29)	12.30(122)

* Figures in parentheses indicate number of samples studied.

Semen smears were prepared for the estimation of morphologically abnormal spermatozoa. Priority was given to the abnormalities in the order listed in table 2 (*i.e.*, a spermatozoan showing both head and tail abnormalities was registered as possessing an abnormal head) in order that the influence of an abnormal spermatozoan would be reflected but once.

RESULTS

The average data for several characteristics and constituents of the semen produced by both groups of bulls at intervals during the 442-day experiment are presented in tables 1 and 2.

The Group II bulls produced slightly larger ejaculates containing a greater total number of spermatozoa than did Group I bulls. No appreciable differences were found in the concentration of spermatozoa or in the proportion of semen constituted by spermatozoa ejaculated by the two groups. The difference in the average spermatozoan volume is largely the reflection of one animal in Group I.

No appreciable differences were found in the initial motility, motility at 100 hours after ejaculation, and livability of spermatozoa of the groups. The improved livability observed in both groups during the colder months may have been a seasonal effect upon this characteristic.

Similar levels of reducing substances, reducing substances in oxidized form, and ascorbic acid were found in the semen obtained from both groups. A marked decrease was observed in the level of reducing substances in oxidized state (which was reflected in the potential reducing capacity) during Period IV.

The initial pH of semen was similar for both groups; however, semen from Group I underwent a greater decrease in pH during incubation than did that of Group II.

The data in table 2 summarize the proportions of the various types of abnormal spermatozoa found in the semen from each group of bulls. Generally, the abnormalities occurred at about the same rate in both groups, with Group I spermatozoa manifesting a greater proportion of the heads of the pyriform type. It will be noted that a greater quantity of morphologically abnormal spermatozoa appeared during Periods III and IV than previously. This may have been effected by the increased rate of semen ejaculation. The differences found between the groups in total abnormalities were attributed largely to one bull in Group I, whose semen contained a characteristically high number of abnormal spermatozoa.

DISCUSSION

In the evaluation of the comparative merits of simple and complex concentrate feeds for breeding bulls, the final conclusion must be based upon the over-all effects of these mixtures upon the character and/or the fertility of the semen produced. Although it has been recognized that no single test presently exists which allows an adequate prediction of the relative fertility of a semen specimen, a combination of tests involving various semen properties and characteristics is believed to contribute valuable information relative to forecasting the impregnating capacity.

In general, the quality of semen produced by the two groups of bulls receiving markedly different concentrate feeds was essentially the same, as determined by various tests. The concentration of spermatozoa in the semen was similar for both groups; however, bulls receiving the complex concentrate mixture yielded ejaculates of larger volume and greater num-

bers of spermatozoa than those ejaculated by bulls consuming the simple mixture. These differences were not regarded as of great importance, since neither group of animals produced semen which was subnormal in these respects. The spermatozoa produced by bulls receiving the two diets possessed similar average degrees of motility and livability, with a slightly higher degree of livability in the semen of bulls receiving the simple mixture during Periods III and IV and in that of bulls receiving the complex feed during Period II.

Various investigators (1, 4, 16) have pointed out the usefulness of the measure of pH change during incubation as an index of semen quality, since this test affords a gross picture of the metabolic activity of spermatozoa, probably involving the effects of spermatozoa numbers, activity and chemical changes. Other studies (1, 10, 17, 20, 30, 31, 35) have demonstrated conclusively the reliability of livability or longevity estimates as forecasters of relative fertility of semen. Because of the strong evidence offered in their support, these two measures were accorded higher recognition as criteria of semen quality than the others used in this study. From this standpoint semen of similar character was produced by the bulls on both feeding programs.

The reducing properties of semen were examined previously and found to be related to the general metabolism of spermatozoa (27, 28, 33) and to states of fertility (5, 27, 28). In view of the results of these investigations, a method was devised for the analysis of semen in which reducing materials probably not measured in previous studies could be accounted for and measured as absolute quantities. No great differences were observed in the quantities of total reducing substances, reducing substances in oxidized form, potential reducing capacity, and ascorbic acid content of the semen of the two groups of bulls.

The small differences observed between the groups relative to the percentage of abnormal spermatozoa were not regarded as important, since both groups appeared to be within a safe range as determined in a very critical examination, and since these differences are explicable on the basis of the consistently high percentage of abnormal spermatozoa shown by one bull in Group I.

Apparently when sufficient energy is provided for growing, breeding bulls, a simple mixture of concentrate ingredients is equivalent to a high protein, complex mixture from the standpoint of the quality of semen produced. Satisfactory growth was found to accompany the production of good semen when the daily digestible nutrient intake was approximately 1.18 lb. per 100 lb. body weight. It is not known whether or not the same results would be found in older bulls or in the same bulls over an extended period of time.

SUMMARY

A study was made of the relative merits of a simple and a complex concentrate mixture when fed with a poor to average grade mixed hay for the production of semen by young bulls during a 442-day experimental period.

Various analyses of 423 ejaculates yielded by the bulls receiving the simple concentrate feed and of 383 ejaculates produced by the bulls consuming the complex concentrate mixture would indicate that good quality semen of similar character resulted from the ingestion of both diets when provided at an average rate of 1.18 lb. digestible nutrients per 100 lb. body weight daily.

Although bulls consuming the complex mixture yielded slightly larger ejaculates containing more spermatozoa per ejaculate and fewer abnormal spermatozoa than those of bulls fed the simple mixture, the decrease in pH upon incubation of semen ejaculated by the latter group was greater than that of the semen produced by the bulls fed the complex concentrate feed.

Regardless of the diet fed, the concentration of spermatozoa in semen, the initial motility, the degree of livability, the size of spermatozoa, the quantity of total reducing substances, reducing substances in oxidized state, potential reducing capacity, ascorbic acid, and the initial pH of semen were similar.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDRO-GUAIARETIC ACID IN DAIRY PRODUCTS. I. ITS ANTIOXYGENIC PROPERTIES IN MILK

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The oxidized flavor is one of the most prevalent off-flavors which develop in market milk. This off-flavor may appear even though the raw milk is of the highest quality and the processing methods are carefully supervised in approved equipment.

REVIEW OF LITERATURE

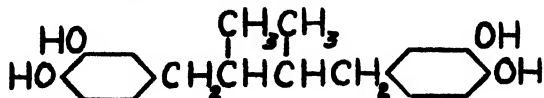
The oxidized flavor in dairy products has been studied extensively, as indicated by the voluminous amount of literature reviewed by Brown and Thurston (4), who cited 412 references.

The compounds or substances proposed as antioxidants for fats are numerous. Matill (9) studied a large number of compounds and found that the active groups of the phenolic compounds were two hydroxyl groups in either the ortho or para configuration. When these groups were in the meta position, the compound did not possess antioxidant properties.

Nordihydroguaiaretic acid (NDGA), one of the compounds which has been used as an antioxidant, was first synthesized in 1918 from hydroguaiaretic acid (12). During cooperative investigations by the United States Department of Agriculture and the University of Minnesota, NDGA was found to occur in a common desert plant, the creosote bush (*Larrea divaricata*), which grows in the southwestern United States (20). Pure NDGA is prepared by crystallization from a crude extract of the plant material.

White, crystalline NDGA is practically odorless but has a slight astringent flavor. It is only slightly soluble in water but is 50 per cent soluble in ethyl alcohol, 20 per cent soluble in propylene glycol, about 15 per cent soluble in glycerol and from 0.3 to 3 per cent soluble in fats and oils (17).

The following chemical formula has been assigned to NDGA (20):



On the basis of Matill's study (9), this phenolic compound would be expected to have antioxygenic properties since the hydroxyl groups are in the ortho position.

Extensive toxicity experiments (5) conducted for over two years indicate that NDGA is entirely harmless (1, 2) in amounts far in excess of

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that required to prevent the oxidation of fat over extended periods of storage.

NDGA has been used successfully in retarding the development of rancidity¹ in lard (6, 7, 8), in bacon (16), and in salt-cured fish (15); in retarding the oxidation of esters of fatty acids (18); in stabilizing carotene in vegetable oil solutions (3, 11); and in retarding oxidative changes in vegetable oils (10) and frozen cream (19).

EXPERIMENTAL METHODS

The milk used in this study was produced by the University dairy herd. In cases where it was important to have milk with little or no metal contamination, the milk was taken directly from the stainless steel milking machines. In other cases, the milk was taken from aluminum milk cans after it arrived at the University Creamery.

Milk samples were scored or criticized for flavor by three or more judges and the consensus taken as the score or criticism. The judges were not aware of the history or treatment of the samples.

Vitamin C determinations were made using the rapid method of Sharp *et al.* (13).

RESULTS

The concentration of NDGA needed for antioxidant protection—the effect of method of adding. Because concentrations of 0.005 per cent were being used successfully in the treatment of fats and oils (1, 6, 7, 8, 10, 11, 15, 16, 18, 19), concentrations of 0.0075 per cent or less, expressed on the basis of the fat content of the milk, were used in this study. The NDGA was added to 4 per cent milk before it was pasteurized at 143° F. for 30 minutes. The development of the oxidized flavor was induced by adding 0.3 p.p.m. copper. The results of a representative trial are found in table 1. The trials were conducted during the period of April through August. A concentration as low as 0.00125 per cent NDGA added either in glycerol solution or in water suspension inhibited the development of the oxidized flavor during 5 days of storage at 40° F. in milk containing 0.3 p.p.m. added copper.

The effect of NDGA on the disappearance of vitamin C in pasteurized milk. The disappearance of vitamin C is reported by Sharp *et al.* (14) to be related to the development of the oxidized flavor in milk. For this reason a series of experiments was conducted to determine whether or not NDGA would retard the loss of vitamin C in milk under normal conditions of storage. Milk was taken directly from the milking machine and samples were prepared containing 0.00125 per cent and 0.0075 per cent NDGA

¹ In other branches of the food industry, the term rancidity usually is used synonymously with the term oxidation. In the dairy industry, oxidation is used to denote oxidative changes in fat, whereas rancidity characterizes hydrolytic changes.

added both in glycerine solution and in water suspension. The milk was pasteurized and cooled. Vitamin C determinations and flavor scores were made every 24 hours. The results of a representative trial are presented in table 2. The trials were conducted during the period of April through August.

The data show that NDGA retarded the destruction of Vitamin C in pasteurized milk stored at 40° F. without added copper. At the end of 96 hours of storage, all of the vitamin C had disappeared in the control samples of the milk which did not contain added copper. At the end of

TABLE 1

The concentration of NDGA needed for antioxidant protection (storage at 10° F.)

Sample no.	Treatment	Flavor comments				
		24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	Control + 0.3 p.p.m. cu.	Sl. cooked Sl. feed	Oxidized 1 ^a	Oxidized 2	Oxidized 3	Oxidized 3
3	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
6	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed

^a The intensity of the oxidized flavor was given values from 1 to 5, as the level of the defect increased.

the same storage period, the loss of vitamin C in the samples which contained NDGA ranged from 54.9 per cent at the lower concentration (0.00125 per cent) to 41.9 per cent at the higher concentration (0.0075 per cent).

Even though the vitamin C disappeared in 24 to 48 hours in the milk which contained 0.3 p.p.m. added copper, the oxidized flavor did not develop during 5 days of storage in the samples which contained NDGA.

The antioxidant retarded the destruction of vitamin C during pasteurization. In the milk which contained no added copper, 18.7 per cent of the vitamin C was destroyed in the control samples during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 6.2 per cent at the higher concentration (0.0075 per cent) to 12.5 per cent at the lower concentration (0.00125 per cent).

TABLE 2
The effect of NDGA on the disappearance of vitamin C in pasteurized milk stored at 40° F.

Sample no.	Treatment	Per cent loss of vitamin C					Flavor criticisms						
		0 hr. ^a	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	18.7 ^b	21.9	25.0	66.8	100	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	0.00125% NDGA	12.5	12.5	15.6	51.6	52.4	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
3	0.00125% NDGA in glycerine	12.5	12.5	12.5	45.6	54.9	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	0.0075% NDGA in water	6.2	6.2	15.6	47.8	41.9	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	0.0075% NDGA in glycerine	9.4	15.6	15.6	40.7	41.9	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
0.3 p.p.m. copper added													
6	Control	56.3	84.4	100	100	100	100	Sl. feed	Sl. feed	Oxi- dized 1c	Oxi- dized 2	Oxi- dized 2	Oxi- dized 4
7	0.00125% NDGA in water	56.3	87.5	100	100	100	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
8	0.00125% NDGA in glycerine	53.2	87.5	100	100	100	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
9	0.0075% NDGA in water	49.9	81.2	100	100	100	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
10	0.0075% NDGA in glycerine	41.8	75.1	100	100	100	100	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed

^a Immediately after pasteurization and cooling to 40° F.

^b Vitamin C content of raw milk, 14.8 mg./l.

^c The numbers 1 to 5 indicate increasing levels of oxidized flavor defect

In the milk which contained 0.3 p.p.m. added copper, 56.3 per cent of the vitamin C was destroyed in the control sample during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 41.8 per cent at the higher concentration (0.0075 per cent) to 53.2 per cent at the lower concentration (0.00125 per cent).

There was no significant difference between the protective effect exerted by the antioxidant which was added in solution and that which was added in water suspension.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will prevent the development of the oxidized flavor during 5 days of storage at 40° F. in whole milk containing 0.3 p.p.m. added copper.

2. In the absence of added copper, the addition of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C in whole milk stored at 40° F.

3. In the absence of added copper, concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C during pasteurization at 143° F. for 30 minutes.

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VITAMIN D CONTENT OF ROUGHAGES¹

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Very little vitamin D generally is considered to exist in the green growing plant. Ergosterol or some other provitamin is present and may be changed over to vitamin D when the plant is cut and exposed to the radiant energy of the sun, as in the sun curing of roughages. From this viewpoint it could be assumed that flue or barn-cured hay would have less vitamin D than sun-cured hay and that little or no vitamin D would be present in roughages cured in the dark or artificially dried with no sun exposure. However, this is not the case according to results obtained at this Station. Assays made on sun-cured and barn-cured hays show practically equal amounts of vitamin D in the two hays. Furthermore, appreciable amounts of vitamin D are present in hays dried without exposure to the sun, as in a dehydrating machine or natural drying in a dark place.

A few trials at other stations have been reported showing that the nutritive and antirachitic values of barn-cured hay compare favorably with that of sun-cured hay for dairy animals, as based on such criteria as rate of growth, physical condition and analyses of certain bones from slaughtered animals. However, no vitamin D contents of the hays were given.

Wylie *et al.* (8), in a trial with yearling heifers, compared the feeding value of barn-cured hay with that of sun-cured hay. The feeding periods extended through three successive winters, each trial being 150 days in length. Each animal received daily 2 lb. of grain, 10 lb. of corn silage and hay *ad libitum*. The heifers in both groups made normal growth with no marked difference in favor of either.

Moore and Thomas (4) report the results of a feeding trial with dairy calves comparing the antirachitic values of field-cured alfalfa hay, barn-dried alfalfa hay and wilted alfalfa silage. They used three groups of dairy calves, six in each group. The calves were first depleted of their body stores of vitamin D and then fed on the above roughages for a period of 6 months. From the results obtained, it was concluded that further fundamental work is necessary. The indications to date are that barn-dried hay and wilted silage will provide sufficient vitamin D for normal functions in growing calves when fed at the usual levels of roughage feeding, *i.e.*, at the rate of 2 to 3 lb. of hay, or the equivalent, per 100 lb. of body weight.

EXPERIMENTAL

In the fall of 1946 the author started a trial with 16 dairy calves (3 days of age) to compare mainly the antirachitic value of barn-cured hay

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with that of sun-cured hay up to first calving. A number of vitamin D assays were made upon the hays, bloods of living animals, and livers of slaughtered animals. At the end of 12 months the animals on barn-cured

TABLE 1
Vitamin D in sun-cured, barn-cured and artificially dried hays

	Av. dry matter	Date sampled	Date assayed	USP units per g. hay		
				First sampling	Second sampling	Third sampling
<i>1946 crop cut 6/27 and 7/1</i>	(%)					
Sun-cured—used in trial	89.85					
1st sampling		6/30 and 7/4/46	8/29/46	0.61		
2nd "		1/6/47	1/24/47		0.34	
3rd "		6/19/47	6/25/47			0.32
" "		"	"			0.61
" "		"	12/2/47			0.55
" "		"	12/16/47			0.43
" " av.						0.48
Barn-cured—used in trial	89.61					
1st sampling		7/26/46	8/29/46	0.51		
2nd "		1/6/47	1/24/47		0.33	
3rd "		6/19/47	6/25/47			0.26
" "		"	"			0.18
" " av.						0.22
Artificially dried (Ardrier)	90.50					
1st sampling		6/27 and 7/1/46	12/16/46	1.14		
" "		"	5/22/47	1.40		
2nd "		3/17/47	3/27/47		0.42	
<i>1947 crop cut 7/14, 7/16 and 7/24</i>						
Sun-cured—used in trial	92.19					
1st sampling		7/16 and 7/25/47	8/13/47	2.00		
2nd "		1/14/48	1/27/48		1.80	
Barn-cured—used in trial	90.50					
1st sampling		8/7/47	8/13/47	2.33		
2nd "		1/14/48	1/27/48		2.00	
Artificially dried (Ardrier)	91.95					
1st sampling		7/14 and 7/16/47	8/28/47	0.59		
2nd "		1/14/48	1/27/48		0.75	

hay were fully equal to those on sun-cured hay in rate of growth, activity and physical appearance. This trial is still in progress and the detailed procedure and results will be reported at a later date. The present paper

deals with the vitamin D content of the various lots of hay handled in different ways. Vitamin D determinations were made according to the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII (5). The vitamin content was calculated by establishing an equation for a curve of response essentially as outlined by Coward (3). Tables 1 and 2 give the USP units of vitamin D per gram of material in the various roughages. The three hays of the 1946 crop were from the same cuttings (table 1). Two lots of hay were harvested and in each case representative samples of the three hays were obtained. Lot I was cut 9 a.m. on June 27; the hay for barn curing was hauled in at 2 p.m. June 28, and the sun-cured hay at 4 p.m. June 29. The weather was sunny except for a shower of short duration on the first day. The material for artificial

TABLE 2
Vitamin D in hays cured in the dark (except no. 7)

Plot	Sample	Date cut		Dry matter	USP units
		(1947)		(%)	(per g.)
A	1	7/3	Plants cut at 5 a.m.	90.92	0.75
	2	7/3	Plants cut at 5 p.m.	91.80	0.54
B	3	8/7	Top of plants, green	91.78	0.84
	4	8/7	Bottom of plants, mostly brown	91.73	1.00
C	5	8/18	Green leaves, hand picked	91.41	0.84
	6	8/18	Brown leaves, hand picked	91.33	1.10
	7	8/18	Hay, whole plant, sun-cured	91.27	2.30

drying was obtained by following the mower and taking a handful of grass every few feet. This was placed in burlap bags so as not to allow any sun exposure. As soon as the mowing was completed, these bags of grass were taken to the barn, chopped and put through the hay drier (Ardrier). The drying involved but a few minutes. The dried material then was spread out on the floor of a dark barn loft for cooling and to complete the drying. Normally a roughage would be wilted until the moisture content was down to around 65 per cent before putting it through the drier. A few days later the material was mixed and sampled. The sample of sun-cured hay was taken from each load as it was hauled in and the barn-dried sample was taken after it had cured, which required approximately 2 weeks.

Lot II was handled in the same manner. It was cut at 4 p.m. July 1; the hay for barn curing was hauled in at 4 p.m. July 2 and the sun-cured hay at 4 p.m. July 3. A small shower occurred during the first night after cutting; otherwise the weather was sunny. The composition of a mixture of these two lots would average approximately 63 per cent timothy and grass, 27 per cent alfalfa, 7 per cent clover and 3 per cent weeds.

Composite samples were made of the two lots each of sun-cured, barn-cured and artificially dried hay. They then were milled and appropriate amounts sent to the assaying laboratory.

The 1947 crop was handled similarly. Lot I was cut at 9 a.m. July 14; the hay for barn curing was hauled in at 11 a.m. July 15 and the sun-cured hay at 2 p.m. July 16. The weather was sunny for the most part and no rain occurred. Lot II was cut at 9 a.m. July 16; the hay for barn curing was hauled in at 10 a.m. July 17. The sun-cured hay was thoroughly soaked with a shower and so it was decided to discard this hay and make another cutting from the same field to obtain sun-cured hay without rain. Another supply for barn curing and artificial drying was obtained at the same time and these samples were mixed with those from Lots I and II. This cutting was made at 9 a.m. July 24; the hay for barn curing was hauled in at 10 a.m. July 25, and since it was a fast-drying day, the sun-cured hay was ready to be hauled in at 4 p.m. on the same day.

Table 1 shows the vitamin D contents of the sun-cured and barn-cured hays used in the calf-feeding trial. Assays for the artificially dried hay harvested from the same lots are included for comparison. Three samples were taken from the 1946 crop of sun-cured and barn-cured hays—one at harvest time or soon afterward, one in January and the last one the following June. The same number will be taken from the 1947 crop. In the 1946 crop there is very little difference in the vitamin D content of the sun-cured and barn-cured hays in the first two samples taken, the units per g. being 0.61 and 0.34 for the sun-cured hay and 0.51 and 0.33 for the barn-cured hay, respectively. The June sample, however, being re-assayed several times, showed greater differences, the sun-cured hay averaging 0.48 and the barn-cured hay 0.22 unit. These results suggest that there may be a tendency for some loss of vitamin D in storage.

The hay which was dried artificially in 1946 had a decided advantage in the amount of vitamin D. The first assay showed 1.14 units per g. and a re-assay of the same sample showed 1.40 units. Another sample from the reserve supply which was stored unmilled from July, 1946, to March, 1947, contained 0.42 unit. These relatively high figures for artificially dried hay were surprising. However, Wallis (7) reported 812 units of vitamin D per pound of artificially dried hay. His sample was cut after dark and dried artificially in a dehydrating machine, thus eliminating any exposure to sunshine. Bechdel *et al.* (1) reported 150 and 300 units of vitamin D per lb. of dehydrated alfalfa hay for two successive seasons. The alfalfa was cut after sundown and dried in an artificial drier to prevent exposure to sunlight after mowing. It was planned to use this hay in a rachitogenic diet for dairy calves, but the amounts of vitamin D were found to be too large. On the other hand, Bechdel and Landsburg (2) found a measurable difference in the antirachitic potency of

dehydrated and sun-cured alfalfa hay when fed to calves as supplements to a basal rachitic diet. Two and one-half pounds of dehydrated alfalfa did not prevent the development of a mild rachitic condition over a 6-month feeding period whereas an equal amount of sun-cured alfalfa served as a complete preventive.

The first two samples of the 1947 crop show higher vitamin D contents in both the sun-cured and barn-cured hays over the previous year. The barn-cured hay was a little higher than the sun-cured hay, the former containing 2.33 and 2.00 and the latter containing 2.00 and 1.80 units. However, the artificially dried hay contained only 0.59 and 0.75 unit per g. Other samples of these hays will be assayed, but to date the barn-cured hay for both years is practically equal to the sun-cured hay in vitamin D content.

The results of the assays on these hays indicate that vitamin D may be present in the growing plant to a larger extent than heretofore has been considered to be the case. In order to throw some light upon this point, several samples of hay were cut from the same field and cured in a dark barn loft, the plants thus receiving no exposure to the sun after being cut. Samples 1 and 2 were cut at 5 a.m. and 5 p.m., respectively, to note any effect the sun might have upon the standing plant (table 2). Samples 3 and 4 were taken to show any differences in the vitamin D content of the top and bottom parts of the plants. The upper part was clipped from the lower without taking any special pains to separate the green stems and leaves from the brown. The lower part, nevertheless, consisted mainly of browned leaves and stems. Samples 5 and 6 were hand picked to obtain only green leaves and brown leaves, respectively, no stems being included. A sample of the entire plants from the same area was sun-cured for comparison. It required around 7 to 10 days for the samples to cure in the dark barn loft. They then were milled and sampled for assaying.

All the samples cured in the dark contained appreciable amounts of vitamin D. They contained lower amounts of the vitamin than did the sun-cured sample, but were higher for the most part than the sun-cured hays obtained in 1946 (table 1). Comparing samples 1 and 2, more vitamin D was present in the morning-cut sample than in the evening-cut hay, even though the standing plants of the latter received 12 hours of sunshine, the respective amounts being 0.75 and 0.54 unit per g. Samples 3 and 4 show a little less vitamin D in the upper than in the lower part of the plants, the respective amounts being 0.84 and 1.00 unit. Thus the brown part appears to contain more of the vitamin than the green part. This also is shown in a comparison of samples 5 and 6, the amounts of vitamin D in the green and brown leaves being, respectively, 0.84 and 1.10 units. The entire plant, as represented by the sun-cured sample 7, contained 2.30 units, which is more than double the amount in either sample 5 or 6 taken at the same time and cured in the dark. This shows the effect of sunshine

in increasing the vitamin D content. The amount in all probability would be higher still if only the leafy portion was considered. Wallis (6) found that the leaves of a good quality green colored alfalfa hay were about six times as potent in vitamin D as the stems. The International Units were 10.45 and 1.72 per g., respectively. Since these hays cured in the dark contained, for the most part, more vitamin D than the sun-cured hays (1946 crop) fed during the first year of the calf feeding trial, one might conclude that they also would provide sufficient amounts of vitamin D to prevent rickets.

CONCLUSIONS

More work needs to be done on the vitamin D content of roughages. The limited results of this study suggest that plants cured in the dark contain appreciable amounts of vitamin D. The brown leaves on growing plants appear to have a somewhat higher vitamin D content than the green leaves. Sunshine plays an important part in the formation of additional vitamin D during the curing process of roughages.

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THE EFFECT OF SOYA-PHOSPHATIDES ON THE ABSORPTION AND UTILIZATION OF VITAMIN A IN DAIRY ANIMALS¹

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It has been shown by various research workers (2, 6, 8, 35) that the liver of the newborn calf contains either little or no vitamin A. Analyses of blood of newborn calves also revealed that the level of vitamin A is exceptionally low (19, 20, 27, 32, 35).

Colostrum, which has been found to be very rich in this vitamin (6, 16, 24, 29, 35), is the first natural material which the calves consume to overcome this deficiency. There is a wide variation in colostrum vitamin A among cows of the same breed (29) as well as in different seasons. Attempts have been made to increase the vitamin A potency of colostrum by feeding extra vitamin A during the latter part of the gestation period. Stewart and McCallum (30) failed to find an increase of this vitamin in colostrum following the feeding of carrots or cod-liver oil. Contrary to this finding, Spielman *et al.* (28) were able to demonstrate an increased amount of vitamin A in colostrum and in the blood and livers of calves following the feeding of large doses of the vitamin during the later stages of the gestation period. Wise *et al.* (35) also found higher levels of vitamin A in both the blood and the liver of the newborn calves when the dams were fed supplementary vitamin A during the gestation period. When large doses of vitamin A or carotene were fed during the later stages of gestation, the decrease in the blood plasma vitamin A and carotene of cows at parturition was not prevented (5, 17, 33, 34), although a higher level was maintained than was observed in the controls.

Numerous reports have been published regarding the importance of vitamin A in calfhood nutrition. Attempts were made to raise calves on skim milk supplemented with vitamin A concentrate (18, 19), usually with disappointing results. Wisconsin workers (19) reported that vitamin A, together with ascorbic acid and nicotinic acid, would increase the survival rate of calves on skim milk. Later work (12, 21) showed no beneficial effect of nicotinic acid feeding, especially in conjunction with large doses of vitamin A supplement.

The question is raised as to whether or not other sources of vitamin A or carotene along with skim milk can be utilized in the same way as the

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colostral vitamin A or carotene. Spielman *et al.* (26) have shown that the carotene of commercial concentrates is poorly utilized by the very young calf. They have reported further that the vitamin A and carotene in colostrum are used more effectively than are vitamin A and carotene added to skim milk or similarly fortified reconstituted skim milk. Knowledge of the exact mechanism of absorption and utilization of vitamin A in the animal system still is inadequate.

Considerable work has been done during recent years regarding the synergism of vitamin E and vitamin A (10, 13) as well as of lecithin and vitamin A. It has been reported that in the case of rats (25), chicks (22), and man (1), the utilization of vitamin A and carotene can be influenced by soybean phosphatides.

The purpose of the present investigation was twofold: (a) To determine the extent to which the vitamin A level of the blood and colostrum of cows and the vitamin A reserve of the newborn calves may be affected by feeding large doses of vitamin A along with soybean phosphatides during the later stages of gestation, and (b) to throw some light on the mechanism of absorption and utilization of vitamin A by calves on a skim milk ration from the time of birth.

EXPERIMENTAL PROCEDURE

This experiment was undertaken during the late winter and early spring months of 1947 while all the animals were on a winter herd ration. Twenty-six pregnant dairy cows of the Jersey and Holstein breeds previously maintained under similar conditions of feeding and management were divided into three dietary groups approximately 30 days prior to parturition. Group I, consisting of ten cows, was again subdivided into two groups of five each. Group I-A received only the usual herd ration³ and Group I-B the same ration plus 10 g. of soybean lecithin⁴ daily. Group II, consisting of eight cows, was given one million I.U. (International Units) of vitamin A⁵ daily, in addition to the herd ration. Group III, consisting of eight animals, received the herd ration plus one million I.U. of vitamin A and 10 g. of lecithin daily.

These rations were continued to the seventh day following the day of parturition. The vitamin A was given orally in gelatin capsules at approximately the same hour each day. The lecithin was mixed carefully with the vitamin A concentrate (fish-liver oil) and special gelatin cap-

³ The herd ration consists of ground corn 400, ground oats 300, wheat bran 100, soybean-oil meal 100, salt iodized 9, and the silage and hay *ad libitum*.

⁴ Soybean lecithin supplied by the American Lecithin Company, Long Island City, New York, contained 70 per cent soya phosphatides (lecithin, cephalin and lipositol) and 30 per cent soybean oil.

⁵ Vitamin A capsules were supplied by the Gelatin Products Company, Detroit, Michigan. One gram of this capsule had 25,000 I.U. vitamin A.

sules each containing 3.33 g. of lecithin and 333,333 I.U. of vitamin A were prepared each week and stored in the refrigerator. Three of these capsules were administered daily to the cows in Group III.

Blood plasma vitamin A and carotene were determined each week before parturition, at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Vitamin A, carotene and lecithin analyses were made on the colostrum and milk samples successively at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Two cows in each of Groups II and III were fed in the same way for approximately 60 days before parturition in order to determine the possible effect of feeding vitamin A for a longer period on the changes of vitamin A and carotene levels in the blood plasma of the cows and their calves and in the liver of the calves as well as in the colostrum and milk.

Blood plasma vitamin A and carotene were determined in the newborn calves before they had access to colostrum or other feed. A few calves from each of the representative groups were slaughtered after birth to determine the liver storage of vitamin A and carotene. All of the calves which were not slaughtered at birth from the above experiment (15 in all) were divided into three groups. There was no predetermined basis for the allotment of the calves to the various groups except that the largest number of calves from cows fed vitamin A and lecithin were allotted to group B, which was assumed to be the group receiving the most rigorous treatment. Preliminary data had shown higher liver storage in the calves from these cows. The calves in Group A were fed colostrum at the rate of 10 lb. per 100 lb. of bodyweight for 7 days after birth. A composite mixture of colostrum was made and stored previously to standardize the feeding in every case. The vitamin A, carotene and lecithin contents of this mixture of colostrum were determined, and the total vitamin A and lecithin consumed by each calf were calculated. The calves in Group B were fed skim milk at the same rate for 7 days after birth. They were not permitted to receive any colostrum. The same quantity of vitamin A consumed daily by the calves in the colostrum-fed group (Group A) was added to the skim milk every day. The calculated quantity of vitamin A oil (25,000-37,500 I.U.) was homogenized with the skim milk before each feeding. Group C was fed skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3-4.5 g.) as was consumed daily by the calves in Group A. This feeding schedule was continued for 7 days following birth. The appropriate quantities of lecithin and vitamin A oil were homogenized with a small amount of skim milk and then mixed with the skim milk to be fed at each feeding. The surviving calves of all the groups were fed whole milk after the seventh day.

Vitamin A and carotene of blood plasma were determined successively at birth, and at 1, 3, 7, 14, and 21 days after birth. After 21 days, some

of the calves from each of the representative groups were slaughtered to determine the total liver storage of vitamin A and of carotene.

Vitamin A and carotene of blood plasma were determined according to the method of Kimble (15). The vitamin A of the blood samples having carotene concentrations exceeding 300 was determined by the method of Boyer *et al.* (3). The vitamin A and carotene of the colostrum and milk samples were determined by the method of Boyer *et al.* (4), with slight modifications. Instead of cold saponification, a hot saponification procedure was adopted. Five milliliters of colostrum or 25 ml. of milk plus 10 g. of caustic potash plus 50 ml. of methyl alcohol were refluxed in a boiling water bath for 10 minutes in a low actinic flask with a ground glass fitted reflux assembly. After cooling, the mixture was transferred to a separatory funnel, rinsing the flask with 55 ml. of water. The mixture was extracted successively with 50-ml. and with 25-ml. quantities of diethyl ether. The remainder of the procedure was the same as that of Boyer *et al.* (4). Liver vitamin A and carotene were determined by using the extraction procedure of Guilbert and Hart (8), with slight modification. Lecithin was determined according to the procedure adopted by Horrall (14). All of the colorimetric measurements were made in an Evelyn Photoelectric Colorimeter, using the appropriate filters.

RESULTS AND DISCUSSION

Effect of prepartal vitamin A and lecithin feeding on the vitamin A and carotene levels in the blood plasma of cows. The individual animal data are too voluminous to report; therefore the data are summarized in table 1. It will be noted that the feeding of lecithin or of vitamin A, or a combination of lecithin and vitamin A, did not prevent a decrease in blood plasma vitamin A and carotene at the time of parturition and beginning lactation. However, higher vitamin A levels were maintained when vitamin A was fed with or without additional lecithin (34). When both vitamin A and lecithin were fed, the highest blood vitamin A level was maintained; the level following parturition was higher (statistically significant) than that found 4 weeks prior to parturition. These data are considered as presumptive evidence that lecithin facilitates the absorption of vitamin A. There was considerable individual variation both in the time when the maximum decrease was noted after parturition and in the magnitude of the decrease. If samples had been obtained at more frequent intervals, perhaps these differences between groups would have been more clear-cut. Feeding of lecithin apparently has a tendency to delay the time of maximum postpartum decrease in blood plasma vitamin A. Although the vitamin A and lecithin feeding was discontinued on the seventh day postpartum, a carry-over effect was still apparent on the twenty-first day, the greatest carry-over effect being observed when both vitamin A and

TABLE 1
The effect of feeding vitamin A and vitamin A plus lecithin on the average concentration of vitamin A and carotene in the blood plasma of cows for the period 4 weeks before parturition to 3 weeks following parturition

Groups	No. of cows	Days prepartum			At parturition	Days postpartum					
		28	21	14		7	1	7	14	21	
Vitamin A in blood plasma (γ 100 ml)											
I-A (No lecithin)	5	20.5	19.1	17.4	18.0	13.7	9.9	13.8	11.5	17.1	18.8
I-B (With lecithin)	5	17.9	18.8	11.9	10.1	12.4	12.4	6.7	15.1	13.7	12.4
II (Vitamin A)	8	19.5	29.6	27.8	26.1	20.4	18.4	21.4	26.0	23.1	22.1
III (A and lecithin)	8	18.9	38.9	38.9	31.2	29.9	25.4	27.5	24.7	26.8	28.7
Carotene in blood plasma (γ 100 ml.)											
I-A (No lecithin)	5	26.5	33.5	32.0	27.3	21.2	15.7	21.2	21.5	24.2	37.1
I-B (With lecithin)	5	30.3	36.8	34.0	34.7	31.8	27.3	30.3	33.1	29.7	30.3
II (Vitamin A)	8	42.2	45.1	29.4	22.2	14.1	16.0	14.1	14.5	17.0	26.3
III (A and lecithin)	8	39.0	42.2	24.0	21.6	15.9	14.5	12.9	9.3	13.5	17.2

lecithin were fed. These data provide additional evidence that high levels of vitamin A in the blood plasma of cows can be maintained by high levels of vitamin A feeding (34) and that this can be done more effectively by feeding a combination of vitamin A and lecithin.

TABLE 2

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the vitamin A content of cows' colostrum and milk up to 21 days postpartum

Cow no.	Vitamin A of colostrum and milk (γ /100 ml.)					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	496	67	35	19	17	18
873J	145	79	21	16	15	11
820J	141	52	33	11	14	15
866J	134	69	68	22	13	14
620H	218	113	29	18		
Mean	226	76	37	17	15	15
Group I-B (control group with lecithin)						
798H	143	46		18	22	25
616H	132	60	58	22	19	17
801H	98	40	21			
763J	216	105	43	27	17	18
800J	134	92	22	25	26	16
Mean	145	69	36	23	21	19
Group II (vitamin A)						
755H	229	167	123	31	25	32
692J	420	131	179	71	23	25
812J	163	172	141	97	14	
711J	304		169	201	74	27
649J	216	222	32	144	28	27
752H	705	302	161	191		
Mean	339	197	134	122	33	28
Group III (vitamin A plus lecithin)						
852H	552	113	175	70	31	24
806H	598	156	195	77	31	39
743H	377	158	112	148	146	22
675J	847	210	125	148	25	40
760J	1119	506	165	162	29	32
Mean	698	229	154	121	52	32

The effect of feeding lecithin alone is not statistically significant although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

The effects of feeding vitamin A and vitamin A plus lecithin on blood plasma carotene at the time of parturition are difficult to interpret be-

cause of the depressing effect (7) of vitamin A feeding on blood plasma carotene. This depressing effect was strikingly shown in the case of three cows fed vitamin A and lecithin for 8 weeks prepartum. The average carotene content of the blood of these cows was 329, 53 and 73 γ per 100 ml. at 8 weeks prepartum and at 7 and 21 days postpartum, respectively.

TABLE 3

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the carotene content of cows' colostrum and milk up to 21 days postpartum

Cow no.	Carotene of colostrum and milk (γ /100 ml.)					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	195	42	18	9	14	17
873J	200	157	17	13	20	19
820J	191	74	36	18	22	28
866J	179	105	52	22	22	9
620H	133	71	18	7		
Mean	180	90	28	14	19	18
Group I-B (control group with lecithin)						
798H	223	53		11	10	14
616H	200	89	46	16	35	8
801H	105	34	17			
763J	218	216	53	16	14	13
800J	172	58	10	8	12	9
Mean	183	90	31	13	18	11
Group II (vitamin A)						
755H	140	108	48	12	8	8
692J	137	36	20	8	8	15
812J	113	62	18	10	7	
711J	152		34	27	8	9
649J	103	69	10	18	7	28
752J	115	47	10	6		
Mean	126	64	23	14	8	15
Group III (vitamin A plus lecithin)						
852H	171	24	15	6	6	6
806H	195	27	25	9	6	12
743H	168	30	16	6	17	7
675J	243	71	26	6	7	15
760J	200	42	16	6	6	6
Mean	195	39	20	7	8	9

Effect of prepartal vitamin A and lecithin feeding on the vitamin A, carotene and lecithin content of colostrum. The data in table 2 show that when vitamin A was fed, the vitamin A in the colostrum was significantly higher than that produced by the groups receiving no vitamin A. When

both vitamin A and lecithin were fed, the vitamin A in the first milking of colostrum was approximately double that of the colostrum from cows receiving vitamin A alone. This difference became less as the milk approached normal and had vanished by the seventh day postpartum. The effects of vitamin A feeding on the potency of the milk still were evident 2 weeks following the end of the vitamin feeding.

The data on the effect of feeding lecithin and vitamin A on the carotene content of the colostrum and early milk are presented in table 3. The feeding of vitamin A depresses the level of carotene in colostrum and milk, a result which has been reported previously (7). It appears also that the feeding of lecithin with the vitamin A enhances this suppressing effect. Additional data are needed to confirm this point.

As previously noted, three animals were fed vitamin A and lecithin for a period of 8 weeks before freshening. The limited data obtained from these animals indicated that higher levels of vitamin A were maintained in the milk after the third day following parturition and the milk carotene was further depressed. These limited data need further confirmation.

The carotene content of colostrum and milk follows the same trend as in the blood. The apparent antagonistic effect of supplemental vitamin A on blood and milk carotene is not explainable in the light of present knowledge; however, its occurrence seems to be beyond doubt.

The total output of vitamin A in International Units per milking is presented in table 4. Although the level of carotene has been depressed in the vitamin A supplemented groups, the total output of vitamin A is higher in these groups. When lecithin was fed with vitamin A, the total output of vitamin A was highest, especially at the first milking. This difference is so great that there is little doubt of its significance. Following the discontinuation of vitamin A supplementation on the seventh day, a sudden marked drop in vitamin A occurred. In the case of those animals receiving both vitamin A and lecithin, the drop was more gradual. Results in the control groups are interesting. Although the vitamin A concentration in the milk of the control group I-A (without lecithin) was higher than that of the control group I-B (with lecithin, see table 2), the total output of vitamin A is higher in the lecithin-fed group. This is due to the greater milk yield. Whether this increased milk-yield is due to lecithin feeding is to be determined by further experiments with a larger number of cows.

The effects of lecithin feeding on the lecithin content of colostrum and milk are shown in table 5. The lecithin content of colostrum and milk seems to be maintained at a higher level when both vitamin A and lecithin are fed. The carry-over effect is still apparent in the milk on the twenty-first day, 2 weeks after the supplemental feeding was discontinued. When

lecithin is fed without vitamin A or vitamin A fed without lecithin, the amount of lecithin in the colostrum and milk is little different from that of the control group which received no supplement. It appears from these data that there may be a reciprocal relationship in the absorption and/or metabolism of these two compounds.

TABLE 4

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in total output of vitamin A per milking up to 21 days postpartum

Cow no.	I.U. ^a of vitamin A per milking					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	136,140	13,557	13,648	6,717	7,541	8,323
873J	16,565	11,831	3,696	3,769	4,482	3,601
820J	17,998	15,731	8,759	4,839	5,723	6,937
866J	9,456	8,361	8,480	5,133	4,732	3,932
620H	50,592	23,273	6,445	4,147		
Mean	46,150	14,550	8,206	4,921	5,620	5,698
Group I-B (control group with lecithin)						
798H	55,600	34,610		9,899	13,075	17,668
616H	51,950	37,710	25,264	11,195	13,078	8,400
801H	87,780	19,854	9,351			
763J	72,350	5,304	19,440	8,629	6,353	7,770
800J	20,512	30,058	5,994	8,146	8,781	3,964
Mean	57,638	25,507	15,012	9,467	10,321	8,950
Group II (vitamin A fed)						
755H	58,883	90,360	47,782	16,685	12,693	16,993
692J	112,493	41,939	60,538	25,285	7,645	12,844
812J	45,740	37,675	45,764	32,017	6,355	
711J	36,652		52,145	67,821	21,279	8,831
649J	34,285	24,123	6,137	41,377	8,436	13,443
752H	280,100	125,412	60,156	72,996		
Mean	94,692	63,002	45,420	42,697	11,282	13,027
Group III (vitamin A plus lecithin)						
852H	102,850	22,056	55,220	19,315	9,649	6,955
806H	455,910	51,539	112,227	33,145	14,169	23,099
743H	295,940	66,164	35,697	59,653	76,830	10,979
875J	146,240	55,198	30,774	42,357	9,092	15,751
760J	141,713	56,997	45,183	51,164	9,417	9,253
Mean	228,530	50,391	55,820	41,127	23,830	13,207

^a One microgram of vitamin A = 4 I.U. Vitamin A. One microgram of carotene = 1.66 I.U. vitamin A.

Effect of supplementing the maternal diet with vitamin A and lecithin on the blood plasma vitamin A and carotene of the newborn calf. The results of this phase of the study are presented in table 6. The plasma vita-

min A of the calves from cows receiving vitamin A was significantly higher than that of those from the control cows. These results are in agreement with those of Wise *et al.* (35) and Spielman *et al.* (27). The

TABLE 5

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the lecithin content of cows' colostrum and milk

Cow no.	Percentage of lecithin in colostrum and milk					
	At partu- rition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I-A (control group without lecithin)						
851H	0.062	0.051	0.049	0.028	0.029	0.024
873J	0.076		0.044	0.039	0.038	0.038
820J	0.058	0.059	0.043	0.038	0.021	0.020
866J	0.068		0.049	0.039	0.029	0.036
620H	0.060	0.080	0.048	0.038	0.034	
Mean	0.065	0.063	0.047	0.036	0.030	0.029
Group I-B (control group with lecithin)						
798H	0.076	0.056			0.042	0.026
616H	0.076		0.056		0.021	0.025
801H	0.051	0.043	0.023		0.042	0.026
763J	0.073		0.074			
800J	0.055	0.053	0.055	0.048	0.026	0.029
Mean	0.066	0.051	0.052		0.033	0.026
Group II (vitamin A)						
755H	0.068	0.063	0.055	0.046	0.031	0.025
692J	0.064	0.046	0.052	0.047	0.024	0.023
812J	0.056	0.046	0.044	0.039	0.036	
711J	0.088		0.074	0.055	0.036	0.040
649J	0.064	0.035	0.026	0.060	0.036	0.038
752J	0.084	0.067	0.059	0.052		
Mean	0.071	0.051	0.052	0.049	0.033	0.032
Group III (vitamin A plus lecithin)						
852H	0.094	0.062	0.057	0.058	0.044	0.036
806H	0.094	0.060	0.050	0.051	0.046	0.051
743H	0.069	0.049	0.040	0.040	0.046	0.030
675J	0.093	0.100	0.180	0.081	0.059	0.055
760J	0.093	0.102	0.075	0.056	0.052	0.051
Mean	0.089	0.075	0.060	0.057	0.049	0.045

slightly higher mean value of the blood plasma vitamin A of calves from cows receiving both vitamin A and lecithin is insignificant.

It appears from the data on blood plasma carotene that a combination of lecithin and vitamin A in the maternal diet exerts a suppressing action on the level of carotene in the blood of the newborn calf. These data, however, are within a range where experimental error of determination is

apt to be rather high, and the difference may not be as significant as the statistic indicates.

The effect of supplementing the maternal diet with vitamin A and lecithin on the liver storage of vitamin A and carotene in the newborn calf. Seven of the calves were sacrificed at birth to determine total liver storage

TABLE 6

The effect of increasing the vitamin A and lecithin content of the maternal diet on the blood plasma and liver storage of vitamin A and carotene in the newborn calf

Dam no.	Calf no.	Blood plasma data		Liver storage data		
		Vitamin A	Carotene	Liver wt.	Vitamin A	Total A
		(γ /100 ml.)	(γ /100 ml.)	(g.)	(γ /g.)	(γ)
Calves from Group I (control cows)						
866J	819J	2.4	4.2			
873J	820J	3.5	6.3			
851H	825H	6.5	11.4			
800J ^a	944J	7.7	4.2			
820J	821J	5.4	9.2	434	0.59	249
620H	948H	8.9	0.0	1000	0.25	252
801H ^a	828H	8.4	5.6	793	0.08	67
	Mean	6.1	5.8		0.31	190
Calves from Group II (cows fed vitamin A)						
755H	905H	7.2	11.4			
812J	946J	9.8	8.5			
711J	823J	8.6	8.4			
853H	830H	9.3	0.0			
752H	829H	10.7	0.0			
692J	822J	11.5	4.9	435	12.0	5241
649J	827J	6.2	7.0	500	13.1	6580
	Mean	9.0	5.7		12.5	5910
Calves from Group III (cows fed vitamin A plus lecithin)						
855J	832J	9.2	0.0			
760J	947J	6.6	2.8			
743H	950H	10.7	0.0			
852H	949H	10.2	0.0			
857H	951H	13.6	0.0			
675J	824J	8.2	5.7	438	22.0	9625
806H	826H	15.1	0.0	945	14.6	13820
	Mean	10.5	1.2		18.3	11722

^a These cows were in Group I-B; lecithin was fed.

of vitamin A and carotene. Three of these calves were from cows in Group I, two from cows in Group II, and two from cows in Group III. As previously reported by other workers (27, 35), a statistically significant greater liver storage was found in newborn calves from dams receiving massive vitamin A supplements. The cows receiving both vitamin A and

lecithin gave birth to calves with almost double the liver vitamin A storage of those from cows receiving vitamin A alone. As can be noted from table 6, this difference was due in part to higher concentration and in part to greater liver weight. Although the numbers are limited, the magnitude of the difference is so great that there is little doubt of the significance, particularly in the light of the other data presented in this paper. Fur-

TABLE 7

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A and lecithin on the plasma vitamin A levels of calves

Calf no.	Dam no.	Dam group	Vitamin A fed daily (I.U.)	Vitamin A in plasma (γ/100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	7.2	4.6	27.9	16.9	9.4	9.3
819J	866J	I-A	25,000	2.4	8.2	24.0	9.9	13.6	8.3
820J	873J	I-A	25,000	3.5	11.9	25.4	23.3	13.5	11.1
944J	800J	I-B	25,000	7.7	13.5	18.2	19.9	14.6	14.1
832J	855J	III	25,000	9.2	11.9	20.2	20.7	18.2	15.9
			Mean	6.2	10.1	23.5	18.2	13.8	11.7
Group B (skim milk plus vitamin A)									
825H	851H	I-A	37,500	6.5	5.3	12.0	Fell sick and died on the 7th day		
946J	812J	II	25,000	9.8	6.6		" " "		
947J ^a	760J	III	25,000	6.6	6.6	8.7	13.9	11.3	6.6
950H ^a	743H	III	37,500	10.7	10.4	12.2	10.1	9.3	22.0
831J ^b	854J	III	25,000	5.9	9.3	13.2	13.3	died on the 11th day	
			Mean	7.9	7.6	11.4	12.4		
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.6	15.7	15.7	18.7	16.8	13.9
949H	852H	III	37,500	10.2	10.7	30.3	35.0	20.8	16.0
951H	857H	III	37,500	13.6	10.7	19.2	27.8	17.2	10.5
830H	853H	II	37,500	9.3	18.3	14.3	28.1	12.2	15.3
829H	752H	II	37,500	10.7	13.6	16.8	22.8	12.7	12.2
			Mean	10.5	13.8	19.3	26.3	15.9	13.6

^a Given 2% coconut oil per feeding.

^b Given lecithin from the 6th day.

ther evidence of the effect of vitamin A feeding on liver storage is shown in table 9. Calves 832J, 831J, and 830H were from dams that received the vitamin A supplement for 8 weeks prior to parturition. When these calves were sacrificed at 21 days of age (calf 831J died at 11 days of age), the liver storage was significantly higher than that of other comparable calves with similar histories and treatment.

Effect of the diet on the blood plasma vitamin A and carotene levels and the liver vitamin A storage of young calves. As previously indicated, the calves from the cows in this experiment were removed from the dams at birth (before nursing) and given special dietary treatments for the first 7 days. These dietary treatments have been described earlier in this paper.

The data obtained are presented in tables 7 and 8. The feeding of colos-

TABLE 8

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A and lecithin on the plasma carotene levels of calves

Calf no.	Dam no.	Dam group	Vitamin A fed daily (I.U.)	Carotene in plasma (γ/100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	11.4	5.7	24.7	38.8	20.6	23.2
819J	866J	I-A	25,000	4.2	6.4	4.9	62.1	49.4	37.2
820J	873J	I-A	25,000	6.3	2.8	26.3	58.7	29.0	21.8
944J	800J	I-B	25,000	4.2	21.7	48.1	22.4	51.0	35.6
832J	855J	III	25,000	0.0	5.7	29.4	35.6	57.0	32.4
			Mean	5.2	8.4	26.6	43.5	41.3	30.0
Group B (skim milk plus vitamin A)									
825H	851H	I-A	37,500	11.4	7.8	13.6	died on the 7th day		
946J	812J	II	25,000	8.5	5.6		" " " " "		
947J ^a	760J	III	25,000	2.8	5.6	7.7	0.0	12.8	5.9
950H ^a	743H	III	37,500	0.0	0.0	3.5	4.9	17.2	21.8
831J ^b	854J	III	25,000	0.0	0.0	0.0	3.5	died on the 11th day	
			Mean	4.5	4.0	6.2	2.8		
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.4	15.3	15.3	2.5	15.3	27.9
949H	852H	III	37,500	0.0	0.0	0.0	4.9	32.4	30.2
951H	857H	III	37,500	0.0	0.0	0.0	4.9	9.6	26.3
830H	853H	II	37,500	0.0	2.6	0.0	2.8	26.3	14.3
829H	752H	II	37,500	0.0	0.0	0.0	3.5	12.8	20.9
			Mean	1.7	3.5	3.1	3.7	19.3	24.0

^a Given 2% coconut oil per feeding.

^b Given lecithin from the 6th day.

trum resulted in a marked increase in the amount of vitamin A in the blood, as has been noted by others (19, 20, 32). There was an increase in blood plasma carotene following the feeding of colostrum (table 8), but the carotene remained low in both the groups fed vitamin A, as was expected. The carotene increase in the latter groups on the fourteenth and twenty-first days resulted from whole milk feeding following 7 days of age. There was no evidence of scours in the colostrum-fed group. The calves receiving skim milk plus the vitamin A supplement did very poorly.

Serious scours developed on the third day. Three of the calves died, two on the seventh day and one on the eleventh day. The calf that died on the eleventh day was from a cow that received both vitamin A and lecithin, and at the time of death this calf had an appreciable liver storage of vitamin A (see calf 831J, table 9). Perhaps the liver storage of vitamin A permitted this calf to endure the rigors of the diet longer than those which died on the seventh day. The other two calves, 947J and 950H, were given 2 per cent cocoanut oil and were able to survive. These calves also scoured from the third to the tenth day, and vitamin A absorption, as indicated by the low blood level, was poor.

TABLE 9

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A plus lecithin on the storage of vitamin A in the liver of calves at 21 days of age

Calf no.	Dam no.	Dam group	Wt. of the liver (g.)	Vitamin A (γ /g.)	Total vitamin A (γ)
Calves from Group A (colostrum-fed)					
819J	866J	I-A	519.6	7.2	3,724.0
820J	873J	I-A	526.8	8.7	4,630.0
832J ^a	855J	III	608.0	53.0	32,244.0
Calves from Group B (skim milk plus vitamin A)					
825H ^b	851H	I-A	988.0	0.15	149.0
946J ^b	812J	II	466.5	11.8	5,524.0
831J ^c	854J	III	453.8	56.3	25,570.0
Calves from Group C (skim milk plus vitamin A plus lecithin)					
823J	711J	II	549.0	35.4	19,470.0
830H ^a	853H	II	1124.0	69.0	77,556.0
829H	752H	II	953.0	36.6	34,900.0

^a The dams got vitamin A or vitamin A plus lecithin for 8 weeks prepartum.

^b Died on the 7th day.

^c Died on the 11th day.

The low level of blood vitamin A in the group receiving skim milk plus vitamin A indicates poor vitamin A absorption on this type of diet. When lecithin was added along with the vitamin A, the blood levels were comparable to those of the colostrum-fed group. These results again indicate that soya lecithin enhances the absorption of vitamin A. There were a few mild cases of scours among the calves in this group, but, in general, they did quite well and were comparable to the colostrum-fed calves in rate of growth and general appearance.

It has been suggested that colostrum vitamin A may be superior in the nutrition of newborn calves to the vitamin A of fish-liver oil or other concentrated sources (9). The data presented herein provide evidence that the higher concentration of lecithin present in colostrum may be partially responsible for the better absorption and utilization of colostrum vitamin A.

An attempt was made to start calves on a skim milk ration plus coconut oil (two calves) and a skim milk ration plus lecithin (two calves). These attempts failed and all the calves died on the fourth day following birth.

The results of this phase of the investigation show that unless adequate quantities of vitamin A or its precursor are present in the ration and unless favorable circumstances for vitamin A absorption are provided, the animal will quickly succumb to vitamin A deficiency, even when there is considerable liver storage.

Limited data on the liver storage of vitamin A determined on the twenty-first day are presented in table 9. Here again it will be noted that the calves fed the vitamin A plus lecithin had appreciably higher liver storages. While the data cannot provide conclusive proof that vitamin A storage is greater when vitamin A and lecithin are fed, rat data (31) have proved this point conclusively.

Previous workers (12) have shown that the feeding of vitamin A in capsules resulted in increased liver storage. The results of the present investigation provide evidence that the storage will be increased still further if lecithin is fed along with vitamin A.

SUMMARY

Twenty-six healthy pregnant dairy cows of the Jersey and the Holstein breeds were divided into three dietary groups approximately 30 days prior to parturition. Each group received the basic herd ration. Group I, consisting of ten cows, was again subdivided into two groups; Group I A received no supplement and Group I-B the herd ration plus 10 g. of soya-lecithin daily. Each of the eight cows in Group II was given one million I.U. of vitamin A (fish-liver oil) daily. Each of the eight cows in Group III was fed one million I.U. of vitamin A and 10 g. of lecithin daily. The supplements were continued up to the seventh day following parturition. Assays of blood vitamin A and carotene and of milk vitamin A, carotene and lecithin were made at intervals up to 21 days postpartum. Blood plasma vitamin A and carotene were determined in all calves, and representative animals were sacrificed at birth to determine vitamin A liver storage.

At parturition the plasma vitamin A level in the control cows fell almost to half of the 4 weeks prepartum level. The level in the cows fed vitamin A supplements remained fairly high, especially for the cows fed lecithin plus vitamin A, indicating that lecithin enhanced the absorption of vitamin A. There was no significant effect of feeding lecithin without vitamin A, although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

Blood plasma carotene was depressed in both vitamin A supplemented

groups. However, when vitamin A was fed for a longer period, the carotene level was depressed still further in the cows fed lecithin along with vitamin A. These limited data indicate that lecithin enhanced the action of vitamin A in depressing the carotene level.

The vitamin A in the colostrum of cows fed vitamin A was greater than that of the control group; when both lecithin and vitamin A were fed, the colostral vitamin A at the first milking was approximately double that of the vitamin A supplemented cows. This shows that lecithin, when added to vitamin A, increased the transmission of colostral vitamin A. The transmission of vitamin A and carotene in milk closely followed the trend found in the blood plasma.

The lecithin content of milk was highest when lecithin was fed to the cows along with vitamin A, and a higher level was maintained in the normal milk. Feeding lecithin without vitamin A had no effect on the transfer of lecithin to milk. When both vitamin A and lecithin were fed, the lecithin of the colostrum and milk was increased.

The blood plasma vitamin A level in the newborn calf was highest and the plasma carotene level was the lowest in the calves from dams fed both lecithin and vitamin A.

The total liver storage vitamin A in the newborn calves from the control group was low (190 γ); it was 5,910 γ in the vitamin A supplemented group and 11,722 γ in the vitamin A plus lecithin supplemented group. Thus, the addition of lecithin to the vitamin A supplement remarkably increased the liver storage.

Three groups of five calves each were fed from birth to 7 days of age as follows: Group A, colostrum; Group B, skim milk plus the same daily quantity of total vitamin A consumed by the calves in the colostrum group (25,000–37,500 I.U. of vitamin A); and Group C, skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3–4.5 g.) available in the colostrum given to Group A.

Every calf in Group B developed serious scours from the third day. Two of them died on the seventh day and a third one on the eleventh day. Their blood plasma vitamin A level was much below that of the colostrum-fed calves. All the calves in Groups A and C grew quite well with slight evidence of digestive disturbance. Blood plasma levels in Group A and C were almost identical, showing the ability of lecithin to increase absorption and utilization of vitamin A.

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CAROTENE AND VITAMIN A IN THE COLOSTRUM OF COWS OF TYPICAL INDIAN BREEDS

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Since the discovery of vitamin A as an anti-infective factor, many workers (3, 8, 14, 15, 18, 21, 24, 27) have studied its importance in the nutrition of dairy calves. It is now recognized that vitamin A is indispensable and must be supplied in adequate amounts during the earlier part of life, since the calf is born with practically no reserve of vitamin A (2, 4, 10, 17). Krauss *et al.* (17) reported a decrease in the incidence of pneumonia in calves which received 15,000 I.U. of vitamin A concentrate daily. Gullickson and Fitch (11), in an experiment involving 72 calves, reported less trouble from digestive disturbances in young calves that were fed cod-liver oil than in calves not given the vitamin A supplement. Phillips *et al.* (24) observed that the administration of shark-liver oil with a high vitamin A potency and certain members of the B-complex eliminated diarrhea and lowered the mortality resulting from pneumonia. Nelson *et al.* (23) recommended the feeding of fish-liver oil as a vitamin A supplement when there was difficulty in raising calves.

Under natural feeding conditions vitamin A supplementation is not usually practiced; however, the value of colostrum as a source of vitamin A for newborn calves has been the subject of investigation by some workers. Stewart and McCallum (30) made an extensive study of the correlation between the incidence of white scours in calves and the vitamin A content of the colostrum. In 83 calves which received colostrum containing more than 250 blue units of vitamin A, only 10.8 per cent developed white scours or allied infections; whereas, in 28 calves which received colostrum containing less than 250 blue units of vitamin A, 25 per cent developed white scours or allied infections. Moore and Berry (22) also have pointed out the significance of adequate colostrum feeding in building up the vitamin A reserve in the calf. Apart from these observations, several papers report that cow colostrum contains more vitamin A than the milk (7, 9, 12, 16, 20). Dann (5) and Kramer *et al.* (16) have shown that cows' colostrum are ten to one-hundred times richer in vitamin A activity than the normal milk. On the first day of life a calf is supposed to receive a supply of vitamin A greater than the later milk can give in 20 to 50 days. Henry *et al.* (13) have noted that the colostrum of first-

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calf heifers is richer in vitamin A than that of cows, although Stewart and McCallum (29) did not note such a difference. However, they did report: (a) The length of the dry period between successive calvings affected the colostral vitamin A. (b) The vitamin A content was independent of season in contrast to that of carotene. The vitamin A content of the colostrum collected from 100 cows varied from 35 to 1,181 I.U. per 100 ml. By the third or fourth day the amount of vitamin A was from one-tenth to one-twentieth of that present immediately after parturition.

The variations in the carotene and vitamin A content of colostral fat from various breeds of cattle have been studied by Gillam *et al.* (9) and Semb *et al.* (26). These investigators have shown that the concentration of carotene and vitamin A in colostral fat is from five to fifteen times that of the fat prepared from normal milk and that these constituents decrease very rapidly during the first week postpartum. Stewart and McCallum (31) were unable to raise the vitamin A content of colostrum of cows on winter feed by feeding 3 lb. of carrots or one-seventh pint of cod-liver oil per day. Spielman *et al.* (28) have studied the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum. Colostrum from cows receiving a low carotene ration for 60 days before parturition contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving a comparatively rich carotene ration. The effect of feed was more pronounced on the carotene content of the butterfat of the colostrum than on the vitamin A content, although vitamin A supplementation for 60 days before calving increased the colostral vitamin A to a considerable extent.

As no comparable data are available for any of the milking breeds of cows in India, it seemed desirable to initiate a study along this line. The results obtained from such a study are presented in this paper.

EXPERIMENTAL PROCEDURE

The colostrum and milk samples were collected from 15 cows in the Institute dairy herd for a period of 8 days postpartum. Nine cows and one first-calf heifer of the Haryana breed and five first-calf heifers of the Sahiwal breed were used. The animals were fed 3.5 lb. of a dairy mixture, 1 oz. of iodized salt and 1 oz. of bonemeal per head daily. The nature of the roughage fed to the cows depended on the season of the year and has been discussed in a previous paper (25).

The colostrum and milk samples were collected each day for 8 days and stored in a refrigerator for subsequent analysis. The percentage composition with respect to fat, solids-not-fat, protein and ash was determined according to the methods outlined in the A.O.A.C. (1). The extraction procedure of Dann (5) was followed for the determination of carotene and

vitamin A. Vitamin A was measured spectrographically in an alcoholic solution of the unsaponifiable matter, and the proper correction for the absorption due to carotene was made. For the conversion of corrected density readings to micrograms of vitamin A, the factor, $E_{1\%}^{1\text{cm}} 328 \text{ m}\mu - 1800$, was used. Carotene was estimated colorimetrically in a petroleum ether solution.

Table 1 gives the data pertaining to the history and breed of the animals used in this experiment.

TABLE 1
Data pertaining to the history of the cows

Animal no.	No. of lactation	Length of dry period	Calving date
Hariana breed			
		(days)	
1	10	166	8-22-42
2	3	180	10-17-42
3	3	76	10-25-42
4	10	144	11-14-42
5	2	146	11-15-42
6	2	149	11-18-42
7	9	287	12-28-42
8	2	148	1-20-43
9	2	37	5-16-43
10	1		5-17-43
Sahiwal breed			
11	1		10-26-42
12	1		1- 3-43
13	1		1- 7-43
14	1		1-17-43
15	1		5-17-43

RESULTS

Composition of colostrum milk. The data on the average daily milk yield and the percentage composition of the colostrum milk with respect to fat, solids-not-fat, protein and ash are presented in table 2. Individual variations are quite apparent. The comparatively lower yields of colostrum and the higher percentages of the above constituents were found mostly in the samples obtained from the Sahiwal heifers, which were considered at one time as being sterile. All of the colostrum samples were characterized by a high percentage of solids-not-fat, protein and ash. The fat content of the colostrum milk from individual cows varied widely from day to day but the percentage of fat in the first two days' samples was lower than in the later milk. The change from colostrum to milk was a gradual one, a fact which has been established by others (6) but, on the whole, the colostrum samples tended to approach normal milk after the fourth day. The protein content was much higher than that usually obtained for the

TABLE 2
Average daily yield and the percentage composition of colostrum

Days after parturition	Yield		Fat		S-N-F		Protein		Ash	
	Range	Av. ^a	Range	Av. ^a	Range	Av. ^b	Range	Av. ^b	Range	Av. ^b
	(lb.)	(lb.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	3-13	6.4	1.6- 8.3	4.37	12.94-24.85	18.37	8.40-18.26	13.16	0.885-1.253	1.028
2	2-14	8.3	0.9- 7.4	4.23	11.16-16.50	14.07	8.55-11.57	9.00	0.711-0.965	0.877
3	4-18	11.2	1.1- 8.1	5.25	9.33-11.9	10.35	5.46-8.26	6.30	0.747-1.043	0.858
4	2-18	11.3	4.3-10.0	5.72	8.43-11.11	9.63	4.01-7.40	5.30	0.711-0.855	0.821
5	2-18	9.5	4.4- 7.4	5.59	8.40-10.2	9.15	4.40-5.10	4.81	0.765-0.920	0.824
6	2-17	8.8	2.1- 8.4	5.17	8.55-10.32	9.18	3.78-5.04	4.46	0.725-0.835	0.790
7	4-19	10.3	4.6- 7.2	5.47	7.39-9.31	8.85	3.90-4.90	4.35	0.736-0.842	0.794
8	4-18	12.0	4.3- 7.8	5.70	9.10-9.37	9.23 ^c	4.62-4.73	4.66 ^c	0.792-0.846	0.812 ^c

^a Average for 15 animals.

^b Average for 9 animals.

^c Average for 4 Sahiwal heifers.

Institute herd milk. Further progress in lactation, however, might cause more diminution in the percentage of protein.

Carotene and vitamin A in colostrum milk. The data showing the variations in the carotene and vitamin A content of the colostrum milk are presented in table 3. The first day's colostrum contained more than four times

TABLE 3
Variations in the carotene and vitamin A content of colostrum milk during the first 8 days of lactation

Days postpartum	Carotene		Vitamin A	
	Range	Av.	Range	Av.
	(γ/100 ml.)		(γ/100 ml.)	
1	33.6-153.9	85.5	63.2-571.8	313.4
2	7.7-160.7	65.1	43.2-500.9	218.6
3	12.1-130.1	49.0	58.3-358.8	204.2
4	17.2-109.0	39.9 ^a	63.1-438.0	157.5 ^a
5	13.6- 70.3	31.1 ^b	58.6-361.4	118.4 ^b
6	12.1- 42.0	23.0 ^b	49.0-107.8	79.5 ^b
7	10.4- 32.3	20.1 ^a	51.5-140.3	77.1 ^a
8	10.8- 43.7	19.7 ^c	47.3-111.6	70.9 ^c

^a Average for 14 animals.

^b Average for 11 animals.

^c Average for 12 animals.

as much carotene and vitamin A as the eighth day's sample, which might be considered equivalent in potency to a sample of milk obtained under pasture conditions. This variation was not so pronounced, however, as that reported by some English workers (5, 13, 29). As compared to the other constituents of colostrum and milk, the drop in carotene and vitamin A obviously is more marked. It might be mentioned also that the first day's colostrum did not always contain more carotene and vitamin A than the samples obtained within 4 days postpartum, although the average values showed consistent decreases. The lower carotene values were found for the animals which received very little carotene in the ration before calving. Some of these animals also secreted correspondingly lower amounts of vitamin A. The wide individual variations that are apparent in this investigation also have been observed by other workers. The average sample of colostrum obtained from the cows on the day of parturition was found to contain 85.5 γ of carotene and 313.4 γ of vitamin A per 100 ml. as compared to 107 γ of carotene and 374 γ of vitamin A for the Cornell University dairy herd on a standard dry-cow ration (28). The vitamin A-carotene ratio remained practically constant during the 8-day experimental period, indicating the flushing of these constituents from the mammary gland after their accumulation during the dry period. The concentration of both carotene and vitamin A decreased to a greater extent during the first 4 days of lactation rather than in the next 4 days. From the standpoint of vitamin A feeding, the samples for the first 4 days assume particular importance. According to Lewis and Wilson (19), the daily intake of vitamin A for a calf should be 11,000 I.U. per 100 lb. of live-weight. On this basis, the ingestion of 3 lb. of an average sample of colostrum during the first 4 days of lactation probably would be adequate for ensuring an appreciable storage of vitamin A in the liver and a satisfactory level of carotene and vitamin A in the blood of the calf. None of the calves born from the above cows showed any signs of vitamin A deficiency. The calves received colostrum *ad libitum*; consequently, the vitamin A supply was satisfactory even though the carotene and vitamin A contents of colostrum were low in a few cases.

Carotene and vitamin A in colostral fat. In order to obtain more detailed information on the carotene and vitamin A contents of the colostral fat, values for each individual cow were determined. The results are presented in tables 4 and 5. Table 4 gives the data on the carotene content of the colostral fat. A marked drop on the second day of lactation occurred in all but two cows (nos. 13 and 15), and a further sharp drop occurred on the third day in all of the cows except no. 4. Thereafter the decline was slow and the carotene level became almost constant by the seventh day. The average first day's colostrum contained seven times as much carotene as the average eighth day's sample. No appreciable dif-

TABLE 4
*Variations in the carotene content of colostral fat during
 the first 8 days of lactation*

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ carotene/g. colostral fat)							
1 ^a	67.5	40.7	25.5	14.6	9.3	6.7	5.6	5.6
2	29.3	19.6	6.4	5.5	3.9	3.3	3.0	
3 ^b	5.7	5.1	4.5	4.6	3.6	3.2	2.3	2.5
4	24.8	8.0	18.6	5.7			6.1	
5	28.5	9.8	4.9				2.7	2.5
6	31.8	22.5	8.0	4.7			2.3	2.1
7	27.9	16.8	8.4	5.2	4.5	4.0	4.1	3.9
8	18.1	10.2	9.8	5.8	5.3	5.3	4.7	3.7
9 ^b	5.4	3.6	2.7	3.5	5.4	5.7	3.3	3.4
10 ^b	20.9	16.2	3.9	3.4	2.6	3.8	3.0	2.9
11	20.2	14.5	8.3	6.8	3.1	3.1	2.0	
12	25.7	15.8	10.6	10.9	7.3	8.8	4.7	2.7
13	14.7	17.1	8.7	7.2	4.1	2.4	3.3	3.9
14	23.1	14.4	8.0	8.1	9.5	5.0	5.2	5.6
15 ^b	8.2	18.0	12.2	8.1				2.4
Av.	23.5	15.5	9.4	6.7	5.3	4.7	3.7	3.4

^a Received comparatively large quantity of green fodders before calving because of the monsoon months.

^b Received very little carotene in the ration before calving because of the drought.

ference was noted between the Hariana and the Sahiwal breeds in regard to their ability to secrete carotene in butterfat. Owing to the small number of animals, the effect of feed on the carotene content of the butterfat could not be studied thoroughly. However, an examination of the data in

TABLE 5
Variations in the vitamin A content of colostral fat during the first 8 days of lactation

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ vitamin A/g. colostral fat)							
1	147.7	77.9	52.0	39.0	28.0	22.0	17.5	16.2
2	72.6	60.0	20.9	17.1	14.3	14.2	12.8	
3	41.0	45.4	52.1	15.8	15.3	15.0	11.7	11.7
4	69.4	43.1	50.0	24.5			22.7	
5	89.1	38.6	23.6				23.0	18.0
6	106.0	75.9	28.1	11.9			11.1	10.1
7	82.9	47.3	29.2	21.1	15.5	11.0	11.0	11.2
8	49.8	25.2	39.7	25.4	18.3	15.1	13.2	12.8
9	30.2	17.4	18.2	17.9	14.2	12.3	13.2	12.7
10	24.3	33.2	29.0	45.0	13.4	9.8	8.9	8.6
11	76.7	60.4	48.6	27.9	24.0	19.2	13.9	
12	94.8	52.0	44.3	43.8	49.5	46.4	15.2	12.3
13	116.7	127.0	56.8	36.3	20.6	13.7	13.0	13.1
14	117.2	78.1	50.5	12.9	13.7	12.6	11.2	12.0
15	80.0	64.5	53.0	25.2				11.0
Av.	79.9	56.4	39.7	26.0	20.6	17.4	14.2	12.5

table 4 reveals that there was no consistent relationship between the carotene intake and the carotene content of the colostrum fat from all the animals except for a few. Animals 3, 9, 10 and 15 secreted very small amounts of carotene, whereas animal no. 1 secreted a comparatively large amount in the colostrum fat. The first four animals were on a carotene-poor ration due to the drought period, whereas the fifth one received large quantities of green feed due to the periodic monsoon. These results tend to show that, in spite of a number of variables, the carotene content of colostrum fat also is affected, like butterfat, by the type of feed.

The data in table 5 show the decrease in the vitamin A content of the colostrum fat during the first 8 days. The average first day's colostrum contained more than six times as much vitamin A as the average eighth day's sample. These results compare favorably with those of Semb *et al.* (26), who observed that this ratio varied from five to fifteen. Although a few of the animals secreted less vitamin A in the colostrum on the first day than on the second, this was not generally the case in subsequent samples. The change in the vitamin A content as a result of the dry ration was not so apparent as it was in the case of carotene. This might be explained on the basis of the relative ease with which carotene is mobilized as compared to vitamin A. Although there was no difference between the average carotene content of the colostrum fat from first-calf heifers and cows, the vitamin A content tended to be higher in the case of the former. It is difficult to say definitely, under the present experimental conditions, whether or not first-calf heifers secrete more vitamin A in colostrum fat than do cows, an observation also made by Dann (5) and Henry *et al.* (13).

Although the numerical values reported in this investigation are not the same as those found by other investigators, there is some parallelism in the findings, especially when due consideration is given to such differences as diet, breed, and environment.

SUMMARY

Colostrum samples from Haryana and Sahiwal cows have been analyzed for the percentage composition of carotene, vitamin A, fat, solids-not-fat, protein and ash.

1. The colostrum contains more solids-not-fat, protein, and ash than does the normal milk.

2. Colostrum contains more than four times as much carotene and vitamin A as milk.

3. Colostrum fat was found to be six to seven times richer in carotene and vitamin A than the fat of normal milk, but both of these constituents decreased markedly during the first week postpartum. The decrease thereafter was relatively slow and carotene appeared to be affected more than vitamin A by the type of ration fed to the animals.

4. The carotene content of colostrum fat of first-calf Sahiwal heifers was comparable to that secreted by the Hariana cows, but the heifers secreted more vitamin A in the colostrum fat than did the cows.

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COMPARATIVE ANTIRACHITIC VALUE OF FIELD-CURED HAY, BARN-DRIED HAY, AND WILTED GRASS SILAGE FOR GROWING DAIRY CALVES

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According to present opinion, hay crops contain little or no vitamin D before they are cut. It is only after the crop is cut and while it is exposed to the rays of the sun during the curing process that activation of certain plant sterols takes place to form vitamin D.

With the newer methods of conserving hay crops, such as curing the hay in the barn or making wilted silage, the time of exposure to the rays of the sun is less than when the crop is cured in the field. Consequently, when barn-cured hay or wilted silage is the sole source of vitamin D for calves, it might be questionable whether they would obtain enough of the vitamin to meet their requirements. A review of the literature does not supply a direct answer to this question.

Some information on the vitamin D content of forage plants subjected to various curing processes, as determined by rat bioassays, is available. Steenbock *et al.* (9) reported that clover leaves cured without exposure to direct sunlight showed no vitamin D activity when fed to rats at the 1- and 5-per cent levels. On the other hand, leaves from the same field that were cured in the sun and fed on the same basis showed definite vitamin D activity. In later experiments Hart *et al.* (4) found that alfalfa hay cured in Colorado with limited exposure to the sun contained some vitamin D but less than hay cured with full exposure to the sun. Russell (7) reported some vitamin D activity in alfalfa leaves cured out of sunlight but considerably less than in leaves cured in the sun or leaves cured in the sun and irradiated. Smith and Briggs (8) reported very little vitamin D activity of alfalfa leaves cured in the dark. Leaves cured for 15 hours in sunlight had considerable activity but not so much as leaves exposed for 57 hours. However, Hodgson and Knott (5) found that an artificially dehydrated pasture mixture of English ryegrass, Italian ryegrass, and white clover from irrigated land had as much calcifying activity as the same material sun cured. Wallis (10) reported a considerable increase in the vitamin D activity of alfalfa hay after sun curing. However, considerable variation was found between crops in this respect, and it was concluded that "there are other influences than the amount of sunshine received which greatly affect the vitamin D content of the resulting hay." One

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sample of hay cut after dark and dried artificially contained 812 I.U. per pound. This value is equal to or greater than some of the values reported for sun-cured hay. Beechdel *et al.* (1) found that artificially dried alfalfa contained considerably less vitamin D than sun-cured hay. These same workers report values of 150 to 300 USP units of vitamin D per pound in two different lots of night-harvested dehydrated alfalfa hay. If such a hay were fed to calves to the extent of their roughage requirements, it should furnish the minimum requirements.

Some experimental data on the antirachitic value of hay for calves have been reported in the literature. Huffman *et al.* (6) found that 2 lb. per day of sun-cured timothy prevented rickets up to 1 year of age and 3 lb. per day cured rickets in a 9-month-old calf. Two pounds of sun-cured alfalfa hay per day prevented rickets in one calf up to 195 days of age. In using the curative method, these workers were unable to obtain a sufficient intake of timothy hay cured in the dark to determine its antirachitic effect. In studying the data of these workers, one comes to the conclusion that about 0.7 lb. of sun-cured hay per 100 lb. of body weight is about a minimum for the prevention of rickets in growing calves.

The Pennsylvania Agricultural Experiment Station (2) found that a mild rachitic condition developed in a 6-month feeding period with 1 lb. of sun-cured alfalfa hay per day added to the basal diet, whereas on the same basis artificially cured alfalfa permitted a severe rachitic condition to develop. Two and one-half pounds of good sun-cured alfalfa hay per day prevented the development of a rachitic condition during a 6-month period, whereas the same quantity of artificially cured hay permitted the development of a mild rachitic condition.

The review of literature gives little information for making practical recommendations on the question of whether vitamin D supplements should be used when barn-cured hay or wilted silage is fed as the sole source of vitamin D for calves. Results reported in the literature on rat bioassays with hays show that hay cured without exposure to the sun contains considerably less vitamin D than sun-cured hay. The data with calves likewise give the same indication. In the experiments thus far conducted with calves, limited quantities of hay were fed to bring out differences between sun curing and artificial curing. This raises the question of whether the artificially dried hay would not have furnished sufficient vitamin D for calves had it been fed according to body weight or the appetite of the calf (2 to 2.5 lb. per 100 lb). For this reason the data on artificially cured hay cannot be used in making practical recommendations for vitamin D supplementation either for artificially cured hay, barn-cured hay, or wilted silage.

The present study was undertaken to determine whether barn-cured hay or wilted silage will supply sufficient vitamin D to growing calves when it

is fed at adequate levels, *i.e.*, at levels which ordinarily would be fed under practical feeding conditions.

EXPERIMENTAL PROCEDURE

In the summer of 1945 a second cutting of alfalfa was harvested simultaneously as field-cured hay, barn-cured hay, and wilted silage. Good weather conditions prevailed during the time the crop was being harvested by the three methods, so there was a maximum exposure to the sun during daylight hours. The wilted silage was exposed for 4 to 6 hours between sunrise and sunset, the barn-cured hay for 12 to 16 hours, and the field-cured hay for 30 to 40 hours, although there was considerable variation in this respect.

Holstein and Jersey male calves were reared to 90 days of age on a ration of skim milk, grain, alfalfa hay and cod-liver oil. In addition, it was necessary to use two crossbred calves, one of which was placed in the Holstein group and one in the Jersey group. Flaxseed jelly, corn meal, or grain were added to the skim milk, beginning when the calves were about 10 days of age, in order to increase the energy intake. Skim milk was discontinued at 30 days of age for the Holsteins and at 45 days for the Jersey calves. Three calves on the experiment (503, 701, 703) received whole milk to 60 days of age, along with alfalfa hay and grain.

At 90 days of age the calves were placed on the basal ration made up as follows: Corn meal, 60 parts; wheat bran, 30 parts; soybean meal, 20 parts; linseed meal, 10 parts; iodized salt, 1 part; calcium carbonate, 2 parts. In addition, 1 lb. of beet pulp per 100 lb. of body weight, 100 g. of dehydrated alfalfa leaf meal, and 4 lb. of skim milk were fed daily. The calves were kept on this ration for a period of 50 days or until they were 140 days of age in order to deplete their vitamin D stores. Calcium, phosphorus and phosphatase values of the blood were used to measure depletion. Following the depletion period the calves were fed, in addition to the basal grain ration, the particular experimental forage they were to receive for a period of 180 days. In some instances it was necessary to place the calves on their respective forages before the end of the 50-day depletion period because of blood values which indicated the incipient stage of rickets. The calves were kept in a darkened barn out of direct sunlight. They were turned to a dry lot for exercise at night.

Groups of six calves each were fed the alfalfa forage cured by the three different methods. Within each group three different levels of forage were fed with two calves on each level (table 1). The Jersey and Holstein calves were distributed equally between and within groups.

The wilted silage was fed on a hay-equivalent basis, taking into consideration the moisture content. The calves received, in addition to the basal grain ration and the specified forage, 4 lb. of skim milk daily. Total

digestible nutrients were fed according to the Morrison standard by adjusting the grain intake after allowing for the T.D.N. in the skim milk and forage. Adjustments of forage and grain were made each 2 weeks.

Two positive control calves were continued on the depletion ration but received 10,200 USP units of vitamin D daily in the form of irradiated yeast after the 50-day depletion period. One negative control animal was used which received the depletion ration but no vitamin D.

After the calves received the forage for 180 days they were slaughtered and the eighth and ninth ribs were saved for ash analysis. Ash determinations were made on the distal 10 per cent of the two ribs after they were subjected to hot alcohol extraction. The calcium, inorganic phosphorus and phosphatase contents of the blood were determined each week, except toward the end of the experiment, when the determinations were made each 2 weeks.

Rat bioassays for vitamin D were made on the forage put up by the

TABLE 1
Rate of forage feeding per 100 lb. of body weight

No. of calves	Field-cured hay (Group 1)	Barn-cured hay (Group 2)	Wilted silage (hay equivalent) (Group 3)
	(lb.)	(lb.)	(lb.)
2	0.5	0.7	0.7
2	1.0	1.2	1.2
2	1.5	1.7	1.7

three procedures in order to obtain comparative values. The usual line test procedure was used by including 10 per cent of the forage in the basal rachitogenic diet.

In 1946 another crop of wilted alfalfa silage was fed to two calves, beginning as soon after birth as the calves would consume the silage. They received a limited quantity of whole milk to 60 days of age, but after this time their sole source of vitamin D was from the wilted alfalfa silage. The silage was fed on a hay-equivalent basis of 1.5 lb. per 100 lb. of body weight. The two calves were slaughtered at 8 and 9 months of age.

RESULTS AND DISCUSSION

The effect of feeding alfalfa cured by the three different methods on rate of growth is shown in table 2. These data show that the best rate of gain was made by the calves on the wilted silage, their average daily gain being 1.71 lb. per day for the 180-day period. The next best gain was by the calves on barn-cured hay, which averaged 1.65 lb. a day, whereas the field-cured hay produced a daily gain of 1.48 lb. Since the feed intake was well-controlled, these results indicate that good gains can be obtained with

TABLE 2

*Gain in weight on field-cured and barn-cured hay and on wilted silage
(180-day feeding period)*

Field cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate ^a	Total gain	Calf no.	Rate ^a	Total gain	Calf no.	Rate ^a	Total gain
	(lb.)	(lb.)		(lb.)	(lb.)		(lb.)	(lb.)
705-H ^b	0.5	330	503-H	0.7	270	703-H	0.7	350
2380-J	0.5	218	2384-J	0.7	288	2379-J	0.7	259
250-H	1.0	300	2557-H	1.2	344	120-H	1.2	330
504-J	1.0	279	2385-J	1.2	266	505-J	1.2	263
330-X	1.5	279	2558-H	1.7	301	701-H	1.7	342
2383-J	1.5	248	332-X	1.7	314	506-J	1.7	282
Total av. gain		267			297			307
Av. daily gain		1.48			1.65			1.71

^a Rate = hay or hay equivalent daily per 100 lb. of body weight.

^b H = Holstein; J = Jersey; X = Crossbred.

wilted silage. There was very little feed refusal by the two calves that were fed at the highest level (an equivalent of 1.7 lb. of hay per day per 100 lb. of body weight). One Holstein calf, weighing 650 lb., consumed as much as 30 lb. of wilted silage per day, which was the sole roughage. The calves on the wilted silage were very sleek in appearance and appeared to do well throughout the experiment.

The results of the ash analyses of the distal 10 per cent of the eighth and ninth ribs are shown in table 3. The results of the analyses of the two ribs were averaged. These results show that all three forages possessed definite antirachitic properties for calves. There does not appear to be any dif-

TABLE 3

The ash values of rib ends

Field-cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate ^a	Ash	Calf no.	Rate ^a	Ash	Calf no.	Rate ^a	Ash
	(lb.)	(%)		(lb.)	(%)		(lb.)	(%)
705-H	0.5	53.1 N ^b	503-H	0.7	50.9 Sl.st	703-H	0.7	53.0 N
2380-J	0.5	55.8 Sl.st	2384-J	0.7	56.6 N	2379-J	0.7	55.0 St
250-H	1.0	56.5 Sl.st	2557-H	1.2	61.1 Sl.st	120-H	1.2	54.9 St
504-J	1.0	56.7 N	2385-J	1.2	56.2 Sl.st	505-J	1.2	59.0 N
330-X	1.5	56.0 N	2558-H	1.7	59.4 Sl.st	701-H	1.7	57.1 Sl.st
2383-J	1.5	61.2 N	332-X	1.7	58.8 N	506-J	1.7	62.0 N

Negative

2570-H

39.0 St

Positive

Positive

2571-H

508-J

50.8 Sl.st

51.6 N

^a Rate = hay or hay equivalent per day per 100 lb. of body weight.

^b N = normal; Sl.st = slightly stiff; St = stiff.

ference in this respect between the three lots of calves that were fed the three different kinds of forage. In all three groups, the calves that were fed at the lowest roughage level showed the lowest ash values. The ash value for the negative control was only 39 per cent and it was necessary to remove this calf from the experiment after 160 days because of the extreme rachitic condition. The ash values for the two positive control animals were not so high as for the calves that were fed the various levels of forage, even

TABLE 4

*The effect of feeding 1.5 lb. field-cured hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
95	8.1	8.1	14.3
102	7.0	6.2	20.6
109	6.7	6.4	18.2
116	7.1	4.8	17.2
<i>Hay added</i>			
122	8.0	5.8	18.2
130	7.8	4.5	17.9
136	8.1	5.8	9.4
147	9.0	6.8	18.8
161	11.3	8.4	12.4
175	11.3	9.3	10.7
189	11.4	9.3	9.9
196	10.3	8.0	9.2
218	10.6	6.4	10.4
231	9.3	6.9	9.9
245	10.5	7.6	9.2
259	10.0	5.8	5.8
274	10.1	7.4	6.6
287	10.4	8.6	5.0
302	10.0	8.7	5.7
316	9.6	8.1	3.9

though the controls were fed 10,200 USP units of vitamin D per day. The calcium intake of these two calves was not so high as for the calves receiving forage, since no extra calcium was fed, yet the intake at the end of the experimental period was as much as 18 g., or well above a 10-g.-minimum. Therefore, forages may contain factors other than vitamin D which aid in calcification.

The calves were examined periodically for evidence of stiffness (table 3). While there was less stiffness or indication of clinical rickets in the group that received field-cured hay, between groups the differences probably are not significant. There did not appear to be a direct correlation between stiffness and the bone ash values. It also was noted that the calves that

showed stiffness did not show abnormal blood values for calcium, inorganic phosphorus and phosphatase at the end of the experiment. During the depletion period, however, abnormal blood values for calcium, phosphorus and phosphatase usually preceded the clinical signs of rickets.

All the detailed data of blood analyses for each calf cannot be presented. However, the data for one crossbred and two Holstein calves that were fed the largest intake on each kind of forage are shown in tables 4, 5

TABLE 5

*The effect of feeding 1.7 lb. barn-dried hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
91	10.9	8.8	8.4
108	11.3	8.0	10.2
115	10.5	6.8	13.9
122	8.5	7.4	14.6
128	7.6	7.7	
136	6.4	8.6	19.4
<i>Hay added</i>			
142	6.3	8.2	16.2
153	8.4	7.4	14.2
167	11.6	8.8	8.4
181	10.8	9.6	7.2
195	11.2	8.9	7.1
210	10.3	8.3	7.3
224	9.6	7.0	6.6
237	9.3	7.8	6.6
251	10.3	6.8	8.0
265	9.8	6.8	9.2
280	9.7	6.4	5.6
293	9.8	8.6	5.0
308	9.5	6.2	7.0
322	9.8	7.0	7.0

and 6. A study of the detailed data does not reveal any marked differences between the three groups of calves. The addition of forage in the three different forms caused the blood values to return to normal following the depletion period.

The quantity of solar radiation received by the forage, as shown in table 7, was calculated from hourly figures covering the period the forage was in the swath and windrow. The values are in terms of gram-calories per square centimeter of horizontal surface. The vitamin D content of the forage as determined by rat bioassays also is shown in the table in terms of International Units of vitamin D per g. of air-dried forage. There does not appear to be any close correlation between the amount of solar radia-

tion and the vitamin D content of the forage, although the vitamin D content in the field-cured hay was somewhat higher.

Using the figure 0.47 I.U. per g. for the barn-dried hay, when the calves were fed at the rate of 0.7 lb. of barn-dried hay per 100 lb. of body weight the intake of vitamin D would be 150 I.U. per 100 lb. of body weight. On the same basis, when the calves were fed at the rate of 1.2 lb. of barn-dried hay per 100 lb. of body weight, the vitamin D intake would be 256 I.U., and when they were fed at the rate of 1.7 lb. the intake would be 363 I.U. per

TABLE 6

*The effect of feeding 1.7 lb. hay equivalent of silage per 100 lb. body weight on blood calcium, phosphorus and phosphatase
(Data on one calf)*

Age	Calcium	Phosphorus	Phosphatase
(days)	(mg./100 ml.)	(mg./100 ml.)	(units/100 ml.)
<i>Basal ration</i>			
101	11.8	9.4	
107	10.5	9.5	
115	11.6	9.1	12.5
122	10.5	8.1	16.4
129	10.6		17.9
136	9.4	8.2	15.0
<i>Silage added</i>			
142	10.8	7.5	16.5
150	10.6	6.8	10.6
156	11.9	7.3	8.7
167	12.1	7.9	6.9
181		8.1	6.3
195	10.8	8.7	8.1
209	12.3	7.4	8.6
225	10.0	7.8	7.2
238	10.9	6.6	6.2
251	9.7	6.3	6.9
265	10.5	6.7	6.1
279	9.7	6.8	7.6
294	9.7	6.9	5.9
307	10.0	7.1	3.7
322	9.7	7.0	7.8

100 lb. of body weight. If 300 I.U. per 100 lb. of body weight is taken as the minimum requirement, it would be met by the feeding of 1.5 to 1.7 lb. of barn-dried hay per 100 lb. of body weight. On this basis, one would not expect marked rickets to develop in the calves fed wilted silage and barn-cured hay in this experiment even though the vitamin D intake was near the minimum allowance or slightly below. The observations on stiffness and bone ash values confirm this opinion.

The two calves that were fed from birth the wilted silage that was made in 1946 showed no evidence of rickets at any time during the experiment.

The calcium, phosphorus and phosphatase values of the blood remained within the normal range. When these two calves were slaughtered at 8 and 9 months of age, respectively, they were in excellent condition and had sleek hair coats. These two calves did not consume as much dry matter from wilted silage up to 90 days of age as they would be expected to consume from hay. However, after 90 days of age they easily consumed the 1.5 lb. (hay equivalent) of wilted silage offered per 100 lb. of body weight.

The vitamin D content of the three forages put up simultaneously in 1946 as determined by rat bioassays is shown in table 7. The figure 0.87 for the wilted silage was checked and found to be correct. Thus, one would not expect the two calves that were fed the wilted silage containing 0.87 I.U. per g. to develop rickets. The high vitamin D content of this lot

TABLE 7

Exposure of forage to solar radiation and its vitamin D content

	Radiation exposure ^a			Vitamin D content (I.U./g.)
	In swath	In windrow	Total	
	(g. cal./cm. ²)	(g. cal./cm. ²)	(g. cal./cm. ²)	
	1945 crop			
Wilted silage	104	117	221	0.56
Barn-dried hay	364	158	522	0.47
Field-cured hay	573	777	1350	0.97
1946 crop				
Wilted silage	134	109	243	0.87
Barn dried hay	323	218	541	0.58
Field cured hay	515	958	1473	0.88

^a Calculated from data furnished by Dr. W. F. Shenton of American University of Washington, D. C.

of wilted silage probably was due to the presence of a larger quantity of foreign material in the crop than was present in the field-cured hay and barn-cured hay. It was necessary in 1946 to use one field which was somewhat weedy in order to have sufficient silage for the planned experiments.

Therefore, the results indicate that barn-cured hay and wilted silage conserved under the conditions of this experiment will contain sufficient vitamin D to prevent rickets in dairy calves when these forages are consumed at adequate levels. The rat bioassay data show that these forages contained less vitamin D than the field-cured hay. The difference, however, was not sufficiently great to precipitate rickets when the forages were fed at the rate of 1.5 to 1.7 lb. of hay equivalent per 100 lb. of body weight.

A greater difference in the vitamin D content of these forages might have been expected in view of the accepted concept of the mechanism of formation of vitamin D in forage crops. However, while the field curing of hay promotes an increase in its vitamin D content, there may be con-

siderable vitamin D present in the crop as it stands in the field. The amount of vitamin D probably is governed by the quantity or area of dead plant tissue, such as dead stems or leaves or partially injured leaves. The amount of dead tissue might vary, depending on such factors as climatic conditions, stage of maturity, disease, and insect injury. Thus, in the case of leaf hopper injury, minute injured areas exist on the surface of the leaf, where activation of the sterols might take place. Probably an absolutely green plant without injury of any sort would contain no vitamin D. However, under practical conditions in the eastern section of the country it is doubtful whether such a condition ever exists.

The suggestion that the vitamin D content of hay crops might be affected by the quantity of dead material in the crop at time of cutting is found in the paper of Bechtel *et al.* (3). For instance, these investigators found that, in the corn plant at the silage-making stage, the silks, tassels, and dried leaves were excellent sources of vitamin D, whereas the green part of the plant was devoid of vitamin D. The effect of some of these factors on the vitamin D content of tissues of forage plants, now is under investigation.

While it seems probable that wilted silage and barn-cured hay contain sufficient vitamin D so that no supplementary feeding of vitamin D is needed for calves kept out of direct sunlight, further fundamental studies on the factors affecting the vitamin D content of plant tissue as it stands in the field at the hay stage need to be carried out. It would seem quite probable that a higher vitamin D intake might be possible where a good quality of barn-cured hay or wilted silage is fed than where a poor quality of field-cured hay is fed, because of the greater palatability of the former.

Rickets has been reported in dairy calves under practical farm conditions where grain was fed in excess so that very little sun-cured hay was consumed. Vitamin A and calcium deficiency also probably would be present where such a feeding practice is being used. It would seem more logical to advocate proper management practices to correct such conditions rather than the addition of supplements to the grain mixtures fed. Limiting the grain fed so that hay consumption could be increased and exposure of the calves to sun would be of benefit. Calves exposed to direct sunlight during the winter months and receiving no other source of vitamin D do not develop rickets at Beltsville.

Vitamin D sometimes is added to commercial grain mixtures for mature dairy cattle. While the cost is low, it is the opinion of the authors that there are not sufficient concrete scientific data at the present time to warrant such a practice. Later developments may justify such supplementation, but scientific fiction is not sufficient justification for such a practice.

SUMMARY AND CONCLUSIONS

1. Wilted silage fed as the sole roughage to growing dairy calves produced gains as good or better than those observed in calves fed barn-cured

and field-cured hay. The calves that were fed the wilted silage were sleek and excellent in appearance.

2. Wilted silage, made in two different years, contained sufficient vitamin D to prevent rickets in growing calves that were kept out of sunlight when the silage was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight on the hay-equivalent basis (3 to 4 lb. per 100 lb. body weight on the silage basis).

3. Barn-cured hay made one year contained sufficient vitamin D to prevent rickets in calves that were kept out of sunlight when it was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight.

4. Rat bioassays of the forages fed for vitamin D, which confirmed the results of the calf-feeding experiment, showed that they contained sufficient vitamin D to prevent rickets in growing calves.

5. While further fundamental data must be collected on the factors affecting the vitamin D content of forages harvested with a minimum exposure to the sun, it seems quite likely that barn-cured hay and wilted silage, at least as conserved under Beltsville conditions, contain sufficient vitamin D for growing calves to prevent rickets if fed at the usual levels of roughage feeding.

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THE EFFECT OF STREPTOMYCIN UPON THE LIVABILITY AND BACTERIAL CONTENT OF BOVINE SEMEN¹

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Various antibacterial agents have been used in efforts to overcome the problems associated with the presence of bacteria in bull semen used for artificial breeding (1, 4, 5, 6). Earlier investigations at this Experiment Station (1) upon the use of penicillin in diluted bull semen showed that certain organisms were resistant to penicillin at levels as high as 2,000 units per ml. Since streptomycin inhibits the growth of a number of organisms which are insusceptible or only slightly susceptible to penicillin, it seemed desirable to study its effect on bacteria commonly found in bull semen.

Guusalus *et al.* (2, 3) have reported that bulls harboring *Pseudomonas aeruginosa* in their reproductive tracts were apt to have low breeding efficiencies and be poor risks for use in artificial breeding. Since Waksman and Reilly (7) have found streptomycin to be bactericidal for *Pseudomonas aeruginosa*, its addition to semen might restore normal breeding efficiency to bulls of lowered fertility known to disseminate this organism in their semen.

EXPERIMENTAL

Effect of streptomycin upon the livability of spermatozoa. In a preliminary study to determine the relative resistance of stored bull spermatozoa to streptomycin, this antibiotic was added to four ejaculates diluted 1:24 with yolk-citrate diluter at levels of 100, 500, 1,000, 1,500, 2,000, 2,500, 5,000 and 10,000 units or γ per ml. of diluted semen. When compared to untreated controls, no marked differences in spermatozoan livability were noted during the 20-day storage period in the levels ranging from 100 to 1,500 γ . However, concentrations of 2,500, 5,000 and 10,000 γ per ml. of diluted semen greatly reduced motility during storage. On the basis of these results, streptomycin was added to ten samples of bull semen at the rate of 100, 250, 500, 750, 1,000, 1,250, 1,500 and 2,000 γ per ml. of diluted semen with appropriate controls. Each of the ten ejaculates was diluted 1:24 with yolk-citrate diluter composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g.

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of sodium citrate dihydrate in 100 ml. of water distilled over glass. The streptomycin powder was dissolved in sterile sodium citrate solution and mixed with egg yolk to provide a diluter with a 1:1 ratio of yolk to buffer.

The diluted samples were stored at 45° C. and the percentages of motile spermatozoa were determined every 2 days for 20 days. In order to avoid bias on the part of the observer making the motility estimations, randomized numbers were placed on the test tubes of diluted semen containing the various levels of streptomycin. Bacterial counts and streptomycin assays were made on these samples after 0, 8 and 16 days of storage.

The ten ejaculates had a mean concentration of 1,054,000 spermatozoa per cubic millimeter, a mean initial motility of 69 per cent active spermatozoa, and a mean methylene blue reduction time of 9.4 minutes.

The mean motility data for the ten ejaculates are shown in table 1.

TABLE 1
The effect of streptomycin upon the livability of bovine spermatozoa
(Mean of 10 determinations)

Streptomycin units per ml of diluted semen	Per cent motile spermatozoa					
	Before storage	After storage at 45° C for				
		4 days	8 days	12 days	16 days	20 days
Control	69	59	44	33	16	10
100	69	57	47	33	19	8
250	69	58	47	34	17	8
500	69	61	47	34	19	5
750	69	59	46	31	18	5
1000	69	59	45	32	15	9
1250	69	59	45	25	14	3
1500	69	60	42	27	13	6
2000	69	60	43	25	14	4

Using the observations made at each 2-day interval, analysis of variance showed no significant differences in spermatozoan livability between levels of streptomycin of 0, 100, 250, 500, 750 and 1,000 γ per ml. However, the three highest levels (1,250, 1,500 and 2,000 γ per ml. of diluted semen) brought about a highly significant decrease in livability as compared to untreated diluted semen.

The relationship between spermatozoan livability and concentration of streptomycin was studied further by means of regression. While both highly significant linear and curvilinear regressions were calculated, a test for significance of departure from linearity showed that a straight line was more applicable to the livability data (fig. 1). Compared to untreated control samples, the mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250 γ of streptomycin.

Effect of streptomycin upon the bacterial content of diluted semen. Bacterial plate counts were determined on nine samples of diluted semen

after 0, 8 and 16 days of storage using veal infusion agar containing 4 per cent sterile defibrinated ox blood. The samples were plated in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 and incubated for 48 hours at 37° C. Desoxycholate agar plates incubated at 37° C. were used for determining the number of bacteria belonging to the coliform group. The same procedure was followed in obtaining bacterial counts on portions of undiluted semen and plain yolk-citrate diluter stored for 0, 8 and 16 days.

The results of the bacterial plate counts are shown in figures 2, 3 and 4. Logarithmic rather than arithmetic means have been used to express the mean number of bacteria in the nine semen samples. Since 1:10 was the lowest serial dilution employed and at least 25 colonies were required at

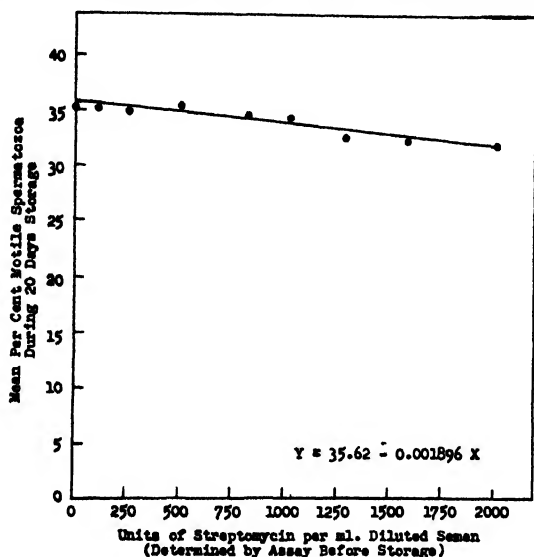


FIG. 1. Relationship of per cent motile spermatozoa during 20 days of storage to level of streptomycin, as shown by regression.

this dilution before a count was considered significant, any counts below log number 2.40 only indicate that the material was not sterile.

As shown in figure 2, levels of streptomycin above 100 γ per ml. were most effective in inhibiting growth of bacteria in freshly diluted semen. Complete inhibition was obtained at all levels of streptomycin in seven of the nine samples. Freshly diluted semen without streptomycin contained an average of 5,000 bacteria per ml., as compared to an average of only 120 bacteria per ml. for the portions of diluted semen containing added streptomycin.

Figure 3 shows that all levels of streptomycin retarded bacterial growth in diluted semen stored for 8 days at 4.5° C. The tubes of untreated

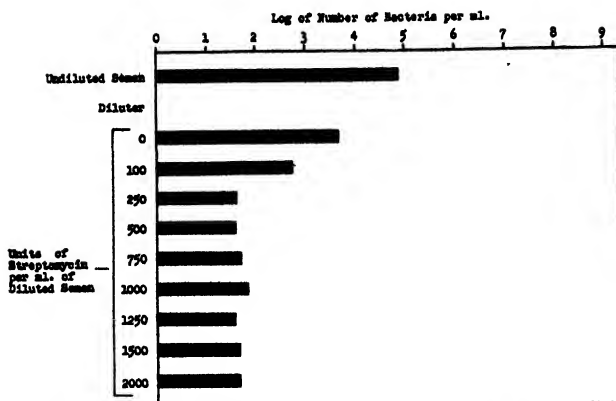


FIG. 2. The effect of streptomycin upon bacterial growth in freshly diluted semen.

semen averaged 82,000 bacteria per ml. while those containing streptomycin averaged 2,000 bacteria per ml. of diluted semen. The high average counts at the 500 and 1,500 γ levels were due to one sample of semen. Possible explanations are contamination during bacteriological analysis or contamination of the individual test tubes of diluted semen with streptomycin-resistant organisms when the tubes were opened for routine motility observations during storage.

Culture plate counts made after 16 days of storage, as shown in figure 4, were rather erratic. In four of the nine diluted samples, minute, pinpoint colonies were present which made counting rather difficult. However, in the remaining five samples streptomycin showed fairly good inhibition of bacterial growth as compared with the controls. The average bac-

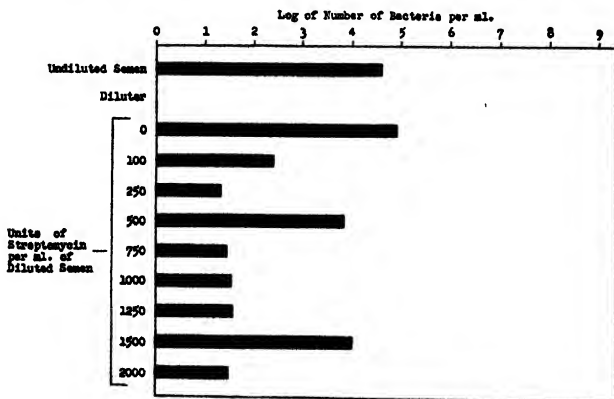


FIG. 3. The effect of streptomycin upon bacterial growth in diluted semen stored for 8 days at 4.5° C.

terial count for diluted semen without streptomycin was 131,000,000 per ml., while the average for diluted semen containing the various levels of the antibiotic was 137,000 per ml. Thus, the antibacterial activity of streptomycin was greatest in freshly diluted semen and semen stored for 8 days.

Very few typical bacteria of the coliform group were present on the desoxycholate agar plates. Only one sample had countable plates and these were present only in the undiluted semen and the tube of diluted semen which did not receive streptomycin. The number of bacteria of the coliform group increased in these two tubes during storage. While there were only a few organisms of this type per ml. in the fresh undiluted semen, counts of 250 and 50,000 per ml. were obtained after storage for 8

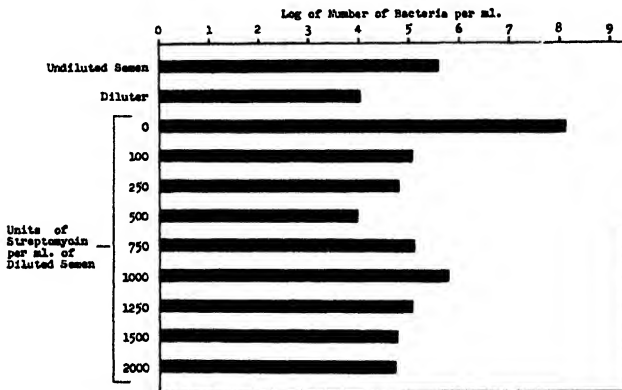


Fig. 4. The effect of streptomycin upon bacterial growth in diluted semen stored for 16 days at 4.5° C.

and 16 days, respectively. The number of coliform bacteria in the untreated diluted semen increased from a few colonies before storage to 5,000 per ml. after 8 days of storage and 1,000,000 per ml. after 16 days of storage.

Total plate counts on the ten samples of fresh, undiluted semen used in the final livability study ranged from 2,000 to 350,000 bacteria per ml., with a mean of 73,000 bacteria per ml. The samples were collected with an artificial vagina and only two of the ten ejaculates had counts exceeding 100,000 bacteria per ml., while seven of the remaining eight had counts of 30,000 or less per ml.

Stability of streptomycin in diluted semen. The stability of streptomycin in diluted semen stored at 4.5° C. was determined by assays at 0, 8 and 16 days with the standard cylinder plate method, using *Bacillus subtilis* as the test organism. The results of the assays made on ten diluted semen samples are presented in table 2. There was no appreciable decrease in the amount of streptomycin over the 16-day storage period.

Studies are now in progress to test the effect of streptomycin upon the fertility of diluted semen used for artificial breeding. Its use in combination with penicillin also is being studied and will be reported as soon as the work is completed.

TABLE 2

*The stability of streptomycin in diluted semen stored at 4.5° C.
(Mean of 10 determinations)*

Theoretical units of streptomycin ^a	Units of streptomycin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
100	96	96	95
250	246	252	247
500	501	520	502
750	814	774	756
1000	1014	1094	988
1250	1282	1250	1267
1500	1586	1511	1529
2000	2012	2065	2014
Diluter alone	0	0	0

^a No. of units expected, based on the total units in the ampules according to the producer.

SUMMARY

1. The additions of 100, 250, 500, 750 and 1,000 γ of streptomycin per ml. of diluted semen did not significantly affect the livability of bull spermatozoa during a 20-day storage period. Levels of 1,250, 1,500 and 2,000 γ per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a storage period of 20 days.

2. A significant linear relationship was found between spermatozoan livability and concentration of streptomycin. The mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250 γ of streptomycin.

3. Streptomycin inhibited bacterial growth in diluted semen as compared with untreated controls. Levels above 100 γ per ml. were especially effective; the greatest antibacterial activity was obtained in freshly diluted semen and diluted semen stored for 8 days. The initial plate counts for ten ejaculates studied ranged from 2,000 to 350,000 bacteria per ml. of fresh, undiluted semen, with a mean of 73,000 bacteria per ml.

4. There was no significant loss in streptomycin activity in diluted semen stored for 8 and 16 days at 4.5° C.

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EFFECT OF ULTRAVIOLET IRRADIATION ON BACTERIOPHAGE ACTIVE AGAINST *STREPTOCOCCUS LACTIS*¹

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Various methods have been advocated for decreasing the incidence of slow acid production due to bacteriophage action during the manufacture of cheese. The control measures have included protection of the mother culture, bulk culture and cheese milk from bacteriophage, and also methods for the destruction of bacteriophage within the cheese plant. Chlorination and irradiation with ultraviolet light commonly have been employed for the destruction of bacteriophage. Since bacteriophage particles frequently are found in the air of the cheese plant, mists containing active chlorine have been used for their destruction. Chlorine mists have the disadvantage of corroding equipment and fixtures within the plant. Use of ultraviolet irradiation for the destruction of bacteriophage would possess various advantages over the use of chlorine compounds, provided it was as effective.

HISTORICAL

Appelmans (2) and Zoeller (10) found that *Shigella* bacteriophage was killed by a short exposure to ultraviolet rays. Mizuno (7) noted that the depth of solution containing bacteriophage, type of suspending liquid, and concentration of bacteriophage influenced the time required for destruction of *Shigella* bacteriophage by ultraviolet rays.

Gates (3) exposed a culture of *Staphylococcus aureus* and its homologous bacteriophage to ultraviolet light and noted a direct relation between the energy required to kill the organism and that needed for inactivation of the bacteriophage; bacteriophage required expenditure of more energy for its destruction.

Sutton (8) exposed various quantities of a bacteria-free filtrate containing bacteriophage active against *Streptococcus cremoris* to ultraviolet rays. When 2-, 4-, and 9-ml. quantities of the filtrate were placed in petri dishes at a distance of 3 inches from a Westinghouse Sterilamp, the bacteriophage was destroyed completely in 6 minutes.

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Luria and Delbrück (5) stated that a suspension of bacterial virus, after inactivation by ultraviolet rays, may have the ability to interfere with growth of a second virus acting on the same host, *Escherichia coli* in this case. A virus inactivated by ultraviolet irradiation inhibited the growth of sensitive organisms but did not lyse bacterial cells.

Anderson (1) conducted experiments to determine whether cells of *E. coli* which had been inactivated by ultraviolet irradiation could be used as hosts for the propagation of bacterial virus. Bacterial cells which were not able to form colonies were able to support growth of virus. Irradiation appeared to reduce the ability of the host to adsorb virus, liberate a virus-inhibiting substance from the host, reduce burst size of the host, inactivate the virus when adsorbed on the host, and kill bacteria.

Whitehead and Hunter (9) stated that bacteriophage active against lactic streptococci could be destroyed by ultraviolet light if the necessary exposure was given. The practical value of ultraviolet light for the destruction of bacteriophage within a cheese plant was considered questionable because of constant reinfection.

Latarjet and Wahl (4) noted that bacteriophage preparations and homologous strains of *E. coli* irradiated separately were not destroyed in the same length of time. Bacteriophage was two to six times more sensitive to ultraviolet irradiation than the homologous strain of *E. coli*. However, when a mixture of bacteriophage and cells was irradiated, the bacteriophage was more resistant.

Luria and Latarjet (6) state that *E. coli* loses its ability to liberate bacteriophage after irradiation, due to inactivation of the intracellular bacteriophage. When bacteria were irradiated between the time of infection with bacteriophage and lysis of the organisms, a rapid increase in resistance was noted. The increase in resistance was thought to be caused by an accumulation of ultraviolet-absorbing materials around the bacteriophage particle. Analysis of the survival curves for bacteriophage indicated that more than one bacteriophage particle grew in one host cell.

METHODS

Source of ultraviolet light. The sources of ultraviolet light were commercial low-pressure mercury-vapor lamps, releasing 85 per cent of their radiations in the range of 2537 Å. One bulb had an output of 279 microwatts and the other bulb an output of 364 microwatts at the surface of the light source.

Determination of effective radiant energy. Measurement of the effective radiant energy of the ultraviolet lamps was made with a Luckiesh-Taylor germicidal light filter in combination with a standard General Electric light meter.

Preparation of bacteria-free filtrates. The bacteria-free filtrates were prepared by adding 1 ml. of a sensitive culture of *Streptococcus lactis* to 100 ml. of sterile skim milk. Bottles containing the sensitive culture were placed in an incubator at 30° C. for 3 hours; then 1 ml. of bacteriophage active against the *S. lactis* culture was added. The bottles containing bacteriophage and a sensitive strain of *S. lactis* were incubated at 30° C. for 48 hours. After incubation, the bottle contents were coagulated with sterile 10 per cent lactic acid, filtered through coarse filter paper, and the resulting filtrate passed through a Selas microporous filter of 03 porosity.

Determination of bacteriophage titer of bacteria-free filtrates. The serial dilution method was used to determine the concentration of bacteriophage in a bacteria-free filtrate. The bacteriophage titer was recorded as the smallest amount of bacteria-free filtrate, in milliliters, which would cause a significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk by a sensitive culture of *S. lactis*.

Irradiation of bacteria-free filtrates containing bacteriophage. A pure culture of *S. lactis* (H1-1) and its homologous bacteriophage (H1-7) were used throughout this series of experiments. The ultraviolet lamps used in the studies were permitted to burn for a period before use in order to stabilize the radiant energy output.

Irradiation experiments were carried out by placing the desired quantity of bacteria-free filtrate containing bacteriophage in a petri dish, distributing the filtrate evenly over the bottom surface and irradiating with the cover removed from the petri dish. Petri dishes having a flat bottom surface were selected for use; they had average inside diameters of 90 mm.

After a bacteria-free filtrate containing bacteriophage was irradiated for a given time, a portion of the filtrate was added to tubes of litmus milk which had been inoculated just previously with a sensitive culture. A significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk, as compared with the control cultures, denoted the presence of active bacteriophage.

RESULTS

Irradiation of 1-ml. quantities of bacteria-free filtrates containing bacteriophage. One-milliliter quantities of bacteria-free filtrates, having bacteriophage titers of 10^{-3} , 10^{-6} , and 10^{-11} , were irradiated with two commercial low-pressure, mercury-vapor ultraviolet bulbs. With each bulb, 1-ml. portions of the bacteria-free filtrates were irradiated at distances of 3, 6, 9, 12, 18 and 24 inches from the source of light. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 1.

The data show that bacteriophage in a bacteria-free filtrate having a titer of 10^{-8} was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of 10^{-6} , when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb. Also, a bacteria-free filtrate having a bacteriophage titer of 10^{-6} was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of 10^{-11} when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb.

The time necessary for destruction of bacteriophage increased as the distance between the ultraviolet bulb and the bacteria-free filtrates containing bacteriophage was increased. This relationship was noted with filtrates having different bacteriophage concentrations and with both ultraviolet lamps.

TABLE 1

Inactivation times during irradiation of 1-ml. quantities of bacteria-free filtrates containing various concentrations of bacteriophage

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
10^{-3}	279	7.5	7.5	10	20	45	90
10^{-3}	364	5.0	7.5	7.5	10	25	35
10^{-6}	279	15.0	30.0	45	75	150	210
10^{-6}	364	7.5	15.0	30	45	75	120
10^{-11}	279	120.0	150.0	270	360	420	900
10^{-11}	364	90.0	120.0	210	300	330	420

The output of radiant energy by an ultraviolet bulb influenced the time required to destroy bacteriophage in a bacteria-free filtrate. Bacteriophage in a bacteria-free filtrate was destroyed in a shorter time by an ultraviolet bulb having an output of 364 microwatts than it was by a bulb having an output of 279 microwatts, when comparisons were made at the same irradiation distance and using filtrates of the same bacteriophage titer.

Irradiation of 2.5 mm. depths of bacteria-free filtrates containing bacteriophage. Quantities of bacteria-free filtrates sufficient to form a layer 2.5 mm. deep in petri dishes and having bacteriophage titers of 10^{-3} , 10^{-7} and 10^{-10} were irradiated with the two ultraviolet bulbs described previously. The irradiation distances were the same as those used for the irradiation of 1-ml. quantities of filtrates. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 2.

The data show that the time necessary for destruction of bacteriophage in bacteria-free filtrates by ultraviolet light was dependent upon the titer of the filtrate. Bacteriophage in a filtrate having a titer of 10^{-3} was destroyed in a shorter time than bacteriophage in filtrates having titers of 10^{-7} or 10^{-10} , when comparisons were made at the same irradiation distance and with the same ultraviolet bulb. Under the same conditions, bacteriophage in a filtrate having a titer of 10^{-7} was destroyed in a shorter time than bacteriophage in a filtrate having a titer of 10^{-10} .

As in the previous experiment, the time necessary for destruction of bacteriophage by ultraviolet light was increased by increasing the irradiation distance. Also, the output of radiant energy by the ultraviolet bulb influenced the time required to destroy bacteriophage in bacteria-free filtrates; the bulb having the greater energy output destroyed bacteriophage in a shorter time under the same conditions.

TABLE 2

Inactivation times during irradiation of 2.5-mm. films of bacteria-free filtrates containing various concentrations of bacteriophage

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
10^{-4}	279	21	26	39	60	70	80
10^{-3}	364	16	20	35	45	55	70
10^{-7}	279	40	60	75	90	90	115
10^{-7}	364	35	45	60	60	75	105
10^{-10}	279	120	135	180	210	225	255
10^{-10}	364	60	75	90	135	180	225

Comparison of irradiation times necessary to destroy bacteriophage in thin and thick films. The data presented in table 1 show that there were only slight differences in the time required for destruction of bacteriophage in a bacteria-free filtrate having a titer of 10^{-3} by two ultraviolet lamps having different energy outputs at irradiation distances of 3, 6 and 9 inches. With irradiation distances of 12 inches or more, there were greater variations in destruction time between the two lamps. The same general relationship was noted with this filtrate (titer 10^{-3}) in table 2.

A comparison of the results obtained with bacteria-free filtrates having bacteriophage titers of 10^{-3} shows that at irradiation distances of 3, 6, 9, 12 and 18 inches, bacteriophage in 1-ml. quantities (thin film) was destroyed in a shorter time than bacteriophage in films 2.5 mm. thick. Similar results were obtained with both lamps. At an irradiation distance of 24 inches, the filtrate having a bacteriophage titer of 10^{-3} was destroyed in less time in a 2.5 mm. depth with one lamp (279 microwatts output) than

it was in a thinner film (1 ml.). With the other lamp (364 microwatts output), bacteriophage was destroyed in a shorter time in a thin film than in a thicker film.

Bacteriophage in a bacteria-free filtrate having a titer of 10^6 (thin film) was destroyed in a shorter time than bacteriophage in a bacteria-free filtrate having a titer of 10^7 (2.5-mm. film) at irradiation distances of 3, 6, 9 and 12 inches but not at 18 and 24 inches.

Bacteriophage in a bacteria-free filtrate having a titer of 10^{10} and irradiated in a 2.5-mm. film was destroyed in a shorter time at all irradiation distances than bacteriophage in a preparation having a titer of 10^{11} and exposed in the thin film formed by 1 ml. of the material.

DISCUSSION

In the trials in which 1-ml. quantities of bacteria-free filtrates were irradiated, the thin films dried after a time, frequently before bacteriophage was destroyed completely. The time required for the moisture to evaporate from the filtrates varied, but on some occasions they appeared dry after 15 minutes. As the filtrate containing bacteriophage dried, the time necessary for destruction of bacteriophage increased appreciably.

The influence of drying on the destruction of bacteriophage by ultraviolet light can be demonstrated by a comparison of data obtained with the filtrate having a titer of 10^{11} in table 1 (1-ml. film) and the filtrate having a titer of 10^{10} in table 2 (2.5-mm. film). The bacteriophage present in the thicker film was destroyed in a shorter time at all irradiation distances than was the bacteriophage in the thin film, which had an opportunity to dry before the end of the irradiation period.

The comparatively long time required to destroy bacteriophage active against *S. lactis* by ultraviolet light at short distances from the lamp indicates that this method of destruction may be of limited value in commercial cheese plants. Since bacteriophage particles are present in the air of cheese plants experiencing difficulty with slow acid production due to bacteriophage, the bacteriophage particles might not be in contact with the rays of an ultraviolet lamp for any appreciable length of time. Certain areas of a cheese plant would be difficult to irradiate, such as the area beneath various pieces of equipment.

SUMMARY AND CONCLUSIONS

Bacteriophage active against *Streptococcus lactis* was destroyed by irradiation with ultraviolet light in a shorter time by a bulb having an output of 364 microwatts than by a bulb having an output of 279 microwatts.

Bacteria-free filtrates having bacteriophage titers of 10^8 were destroyed in a shorter time than filtrates having higher bacteriophage titers.

Increasing the distance between the ultraviolet bulb and the bacteria-

free filtrate resulted in an increase in the time required to destroy bacteriophage in the filtrate.

Bacteriophage in a thin film of bacteria-free filtrate was destroyed in a shorter time than bacteriophage in a thicker film, provided that the thin film did not dry before the bacteriophage was destroyed. Drying appeared to make bacteriophage significantly more resistant to destruction by ultraviolet light.

The long time necessary to destroy bacteriophage by ultraviolet light at relatively short distances from the lamp and the increased resistance of dry bacteriophage to ultraviolet light appear to make this procedure of doubtful value for the destruction of bacteriophage in commercial plants experiencing difficulty with bacteriophage.

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THE KEEPING QUALITY, SOLUBILITY AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS. I. KEEPING QUALITY¹

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The volume of whole milk powder manufactured in the United States greatly increased during World War II. This product has not always been well accepted because under certain conditions it is subject to chemical or physical deterioration which may affect its palatability and reconstitutability in a relatively short period of time.

Many of the basic factors involved in the appearance of storage defects in whole milk powder are well known. However, further study is necessary to increase existing knowledge of the causative factors and thus to increase the shelf-life and consumer acceptance of whole milk powder.

REVIEW OF LITERATURE

A review of the literature indicates that high preheating temperatures of milk improve the keeping quality of powdered whole milk. The treatments reported as giving the best results varied considerably in the temperature-time ratio employed. The following heat treatments have been reported as beneficial in the production of whole milk powder of good keeping quality: 170 to 181° F. for 30 minutes (10, 11, 13, 24); 175° F. for 15 minutes (15); 190 to 195° F., without statement regarding time of exposure (20); 190° F. for 20 seconds, followed by a holding period of 2 to 3 minutes at a slightly lower temperature (17, 22); 220° F. for 10 seconds (15); and 250° F. for 1 second (24).

The reasons given for the effectiveness of the high preheat treatment by the above workers included: (a) the production of reducing compounds, namely sulphydryls, and (b) more complete inactivation of enzymes. The

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production of sulphydryls through high preheat treatment is indicated by the work of other authors (6, 7, 16).

It has been shown that lecithin is easily oxidized, yielding a tallowy flavor (16, 23). In 1902 it was reported that temperatures of 203 to 230° F. destroyed as much as 26 per cent of the lecithin in milk (1), which may partially explain the improved keeping quality of whole milk powder made from high preheat-treated milk. Separate preheat treatment of cream and skim milk is associated with the fat and more particularly the lipids (20). Further evidence that lecithin may, in part, be responsible for the development of oxidized flavor has been produced by removing approximately one-half of the lecithin prior to powdering, which resulted in a better keeping powder even though the preheat treatment was only 160° F. for 30 minutes (2, 3).

Vacuum condensing of the milk improved the keeping quality of the powdered whole milk, apparently by removal of volatile catalysts (12). Condensing the milk to a high concentration was reported as desirable in the production of better-keeping powdered whole milk (24), and an increasing concentration of the milk—31, 38 and 45 per cent total solids—was shown to result in less retention of oxygen by the powder and an improved keeping quality (9).

All of the literature is in agreement that a low storage temperature improves the keeping quality of whole milk powder (4, 5, 8, 10, 14, 15, 17, 18, 19, 21). For instance, it was shown (4, 5) that, although little difference in the keeping quality of the whole milk powder resulted when the storage temperature range was 39.2 to 53.6° F., a very marked impairment of the keeping quality was evidenced in powders stored at 98.6° F. A study (14) with whole milk powders containing 1.54 per cent moisture showed an improvement in keeping quality as the storage temperature was progressively decreased from 86 to 38.4° F. A straight line relationship was indicated in the range of 77 to 50° F. Below 50° F. the improvement in keeping quality was more marked for each 9° F. decrease in temperature, and at 38.4° F. the rate of oxidative deterioration was one-half that found at 77° F. Lea *et al.* (17) considered storage for one day at 59° F. equivalent to 6 hours at 98.6° F. or 3 hours at 116.6° F.

EXPERIMENTAL PROCEDURE

The variables in the manufacturing procedure used in this study were limited to preheat treatment, precondensing and storage temperature. The four preheat-treatment levels studied were: 160 and 170° F. for 30 minutes and 170 and 180° F. for 10 minutes. For each run, morning milk in about 158-lb. quantities was obtained from the college herd and as nearly as possible from the same cows each day. Milk preheated at one of the levels indicated above was mixed thoroughly and divided into two equal parts.

One-half was then precondensed to approximately 20 per cent and the other to approximately 40 per cent total solids. The concentrated milk was homogenized at the condensing temperature and at 2,000 lb. pressure by means of a C. P. Multiflo homogenizer of 125-gallon capacity. The milk powder obtained from each lot of the concentrated milk was divided further into two lots and stored at 45 and 100° F.

The concentrated milk was atomized by air under 60 lb. of pressure and dried in an experimental spray drier. The spray nozzle was 1 mm. in diameter and was centered in the air outlet of 2 mm. diameter. The drying air, at about 255° F., flowed concurrently with that of the spray. The moisture-laden air was drawn from the drying chamber at approximately 160° F. through cloth dust collectors and then through a spray of cold water for dehydration by cooling.

The moisture content of the powder was determined by the vacuum oven method. Extremes in moisture content were 1.6 to 3.1 per cent, with most of the samples containing between 1.8 and 2.6 per cent moisture.

Immediately after drying the powder was manually mixed by means of a large spoon; 120-g. quantities were air-packed into no. 2 flat tins, hermetically sealed and stored at 45 and at 100° F. The powder was reconstituted on the basis of 1 part of powder to 7 parts of water and was scored by a panel of four judges 1 day after manufacture and at intervals of 1, 2, 4, 5, 6 and 10 months thereafter. For flavor scoring a previously unopened can was used. The following arbitrary scale of numerical values was used to rate the flavors: 1-2, bad; 3-4, poor; 5-6, fair; 7-8, good; 9-10, excellent. The "excellent" rating was awarded to samples free of any off flavors except for slight heated flavor. Whenever a heated flavor was detectable, the reconstituted milk was found not to be oxidized. The "good" rating was given to milk which was still quite acceptable in flavor, although it might have a very slight taint which could not be readily defined. The remainder of ratings were based on degrees of oxidized flavor.

RESULTS AND DISCUSSION

The changes in average flavor scores of the milk powder samples are shown in table 1. The effects of preheat treatments on the retention of palatability in air-packed milk powder as brought out here are in general agreement with the effects observed by other investigators. The powders made from milk preheated at 170° F. for 10 and 30 minutes and at 180° F. for 10 minutes deteriorated little in flavor during a 10-month period of storage at 45° F., but powders made from milk preheated at 160° F. for 30 minutes deteriorated in flavor rather rapidly during storage at 45° F. Pre-heat treatment of the milk at 170° F. for 10 minutes is not as effective as the more drastic preheat treatments in inducing good keeping quality in the powder, and treatment at 160° F. for 30 minutes is very ineffective. When

stored at 100° F., all of the powders deteriorated rapidly in flavor and became oxidized in flavor in 1 to 2 months.

Hetrick and Tracy (9) found that increasing pre-concentration of the milk (31, 38 and 45 per cent total solids) resulted in powders of better

TABLE 1
Average flavor changes during storage

Powder from 20% concentrate			Powder from 40% concentrate		
Age	Av. flavor score after storage at		Age	Av. flavor score after storage at	
	45° F.	100° F.		45° F.	100° F.
Preheat treatment—160° F. for 30 min. ^a					
1 day	7.7	7.7	1 day	8.3	8.3
1 mo.	7.2	5.7	1 mo.	7.1	5.9
2 mo.	8.0	4.3	2 mo.	7.7	5.0
4 mo.	7.0	2.8	4 mo.	7.0	3.2
5 mo.	6.4	2.5	5 mo.	6.1	2.4
6 mo.	7.0	3.1	6 mo.	6.3	2.3
10 mo.	5.5		10 mo.	5.4	
Preheat treatment—170° F. for 10 min. ^b					
1 day	8.0	8.0	1 day	7.9	7.9
1 mo.	8.1	6.2	1 mo.	8.4	7.0
2 mo.	8.3	4.9	2 mo.	7.9	5.4
4 mo.	8.0	3.3	4 mo.	7.7	3.9
5 mo.	7.9	3.6	5 mo.	7.8	4.6
6 mo.	7.7	2.5	6 mo.	7.5	3.6
10 mo.	7.2		10 mo.	7.3	
Preheat treatment—170° F. for 30 min. ^a					
1 day	7.8	7.8	1 day	8.1	8.1
1 mo.	8.1	6.2	1 mo.	8.1	7.1
2 mo.	8.2	6.1	2 mo.	8.3	6.5
4 mo.	7.6	3.1	4 mo.	7.8	4.6
5 mo.	7.6	3.3	5 mo.	7.8	6.5
6 mo.	8.0	3.3	6 mo.	8.1	5.0
10 mo.	7.8		10 mo.	8.2	
Preheat treatment—180° F. for 10 min. ^a					
1 day	8.3	8.3	1 day	8.6	8.6
1 mo.	8.3	6.6	1 mo.	8.3	7.3
2 mo.	8.1	5.9	2 mo.	8.3	6.9
4 mo.	7.8	3.6	4 mo.	7.8	4.9
5 mo.	7.9	3.7	5 mo.	8.1	4.7
6 mo.	8.0	3.2	6 mo.	8.1	4.4
10 mo.	7.8		10 mo.	8.2	

^a Each score is the average of 6 samples.

^b Each score is the average of 4 samples.

keeping quality. From the results reported in the present paper, it appears that precondensing to 40 per cent total solids and preheat treating at 170° F. for 30 minutes or 180° F. for 10 minutes yields a powder with slightly better retention of palatability than does powder made from milk precon-

densed to the 20 per cent total solids level. The effect of concentration on the keeping quality was more marked when the powder was stored at 100° F. (table 1). However, at this temperature the improved keeping quality does not appear to be commercially significant.

CONCLUSIONS

1. Preheat treatment of the milk at 170° F. for 30 minutes or 180° F. for 10 minutes resulted in powders of good palatability even after storage, air-packed, for 10 months at 45° F. Preheat treatment at 170° for 10 minutes was slightly less effective for producing powders of good keeping quality. Air-packed powders made from milk preheated at 160° F. for 30 minutes deteriorated rapidly when stored at 45° F.

2. Milks preheated at 170° F. for 30 minutes or at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids resulted in powders which retained slightly better palatability during 10 months of storage at 45° F. than did powder made from the milk precondensed to approximately 20 per cent total solids.

3. All air-packed powders stored at 100° F. rapidly became oxidized in flavor. The powders made from milk preheat treated at 170° F. for either 10 or 30 minutes and at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids deteriorated in palatability slightly less rapidly than did the powders made from the 20 per cent preconcentrate.

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BENEFICIAL EFFECT AND ECONOMIC IMPORTANCE OF USING ALL COLOSTRUM PRODUCED IN CALF RAISING

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The high value of colostrum in the nutrition of the newborn calf has been well established (2), yet on most farms the colostrum not nursed by the newborn calf is wasted or not used for calf feeding. Practical methods of colostrum utilization must be devised and demonstrated to encourage its general use in raising herd replacements. This paper reports such a practical method.

Dann (4), in 1933, reported the vitamin A content of colostrum to be many times that of normal milk. Since then research workers have demonstrated repeatedly the importance of vitamin A in the nutrition of the newborn calf (5, 6, 8, 9, 10, 11, 13, 18). Colostrum also has been reported to be high in riboflavin (12, 15, 16), and a recent paper by Wiese *et al.* (17) indicates the newborn calf must have a dietary source of this vitamin.

In a recent paper on the physiological effects of extending the colostrum feeding period to seven days, the blood plasma level of vitamin A was reported to increase rapidly following the ingestion of colostrum, reaching a peak on the seventh day (14). The calves fed extra colostrum made more rapid weight gains and showed no signs of digestive disturbances during the colostrum feeding periods. The economic advantages of utilizing all colostrum in calf raising also were pointed out.

During the previous experiment, surplus colostrum was frozen and stored until used. On the average dairy farm this method for utilizing colostrum is not practical, since few farms are equipped to refrigerate and store any quantity of colostrum. Considerable inconvenience also is encountered in the feeding, since extra time and effort are required to prepare the colostrum for feeding.

The investigation presented in this report was undertaken to determine the effects of intermittent colostrum feeding for the duration of the milk feeding period. Observations were made of the effects on blood plasma vitamin A and carotene, weight gains and physical performance as indicated by condition of the animal. This experiment also demonstrates a practical method of colostrum feeding that could be followed on most dairy farms.

EXPERIMENTAL PROCEDURE

Calves born in The Ohio State University dairy herd between November 30, 1945, and December 1, 1946, were divided into two groups at birth.

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The five major dairy breeds and both sexes were represented within the groups. The calves were not permitted to nurse. During the first 3 days they were nipple-pail fed colostrum from their dams at a rate of 10 per cent of their body weights. After 3 days, the control group (Group II) was fed Holstein milk at the following rates: 10 per cent of body weight for the first 3 months, 8 per cent of body weight during the fourth month, 6 per cent of body weight during the fifth month, and 4 per cent of body weight during the sixth month. The group receiving extra colostrum (Group I) was fed according to the same schedule except that part or all of the Holstein milk was replaced by colostrum when available. The term colostrum as used in this experiment refers to the production of the first 3 days immediately following parturition. No effort was made to store any of the colostrum and it was used completely at each milking. A concentrate mixture, mixed hay of average quality and water were provided *ad libitum* to all calves up to 3 months of age. After 3 months of age the calves were fed the concentrate mixture twice daily and the amount increased until each calf was receiving approximately 4 lb. daily at 6 months of age.

All calves were weighed at birth, on the third day, and at weekly intervals during the experimental period. Calves were bled for vitamin A and carotene analyses at the time of weighing during the first 4 weeks. After that, the calves were bled at the end of each succeeding 4-week period. Blood samples were drawn according to a definite schedule without consideration of the amount of colostrum fed during the interval between bleedings. Blood plasma analyses for vitamin A and carotenoid pigments were determined by the method of Kimble (7). An Evelyn photoelectric colorimeter with appropriate filters was used for each determination.

EXPERIMENTAL RESULTS

A total of 76 calves, born in a 1-year period and made up of 19 Ayrshires, 17 Guernseys, 16 Jerseys, 15 Holsteins and 9 Brown Swiss, was used in this experiment. Twenty-two of the 36 calves starting the experiment in Group I were on experiment the full 24 weeks. Of the 40 calves in Group II at the beginning, 20 remained at the end of the 24-week period. The decline in numbers was primarily a result of selling bull calves and is without serious variation either in groups or breeds. Although a number of calves in both groups was afflicted with minor cases of scours, only 1 calf left the experiment as a result of calfhood diseases. This calf, a member of Group II, died of pneumonia.

During the experimental period 78 cows dropped calves and produced a total of 5,772 lb. of colostrum, or an average of 74.0 lb. per cow. Thirty per cent of the colostrum produced was used in feeding the calves during the 3-day period immediately following birth. The surplus, over 2 tons,

was used in feeding calves in Group I. Not all the colostrum used in this experiment was produced by the above cows, since colostrum feeding extended approximately 24 weeks beyond the time the last calf was allotted to the experiment. However, colostrum was produced during all seasons of the year and under both pasture and standard winter feeding conditions.

Table 1 presents the data on the amount of colostrum fed per calf during the first 3 days and for each succeeding 4-week period. The number of calves included in each average is tabulated. There is a wide individual variation in the amounts of colostrum received by the calves in Group I. One calf received as much as 445 lb., while another in the same group received as little as 50 lb. The variation in amount fed per calf resulted from the variation in calving dates and the sale of calves.

Data on the sex distribution, average weekly gains, and cumulative weight gains by groups are presented in table 2. It will be noted that the calves of Group I made more rapid and consistent weight gains during the

TABLE 1

Average amount of colostrum fed to each calf receiving extra colostrum for the first 3 days and by 4-week periods thereafter

	First 3 days ^a	4-week period					
		1	2	3	4	5	6
Lb. of colostrum fed	25.7	43.8	34.0	33.2	32.4	22.7	37.2
Av. no. of calves	36	35	32	26	25	19	17

^a Each control calf received 25.2 lb. of colostrum during the first 3 days.

first 4 weeks and maintained that advantage throughout the experiment, even though the number of male calves is greater in the Group II. Another important observation was that the calves in Group I exhibited a more thrifty appearance as indicated by their alertness, quality and gloss of hair coat, and greater vigor. Although a regular weighing schedule was followed, the variation in weekly gains was to be expected because feed and water were given *ad libitum*.

The average data on vitamin A and carotene changes in the blood plasma for each group and for breeds within the groups are presented in tables 3 and 4. The blood plasma levels for both carotene and vitamin A are extremely low at birth. This is in agreement with previously reported experiments (10, 14). The substantial increases in the blood plasma vitamin A and carotene noted on the third day are attributed directly to colostrum feeding. The early peak levels of vitamin A reached on the third day were consistent for both groups. These levels were not exceeded in either group until the sixteenth week. In Group II the early peak level of carotene in the blood coincided with the early peak level for vitamin A, but in Group I the early peak level for carotene was not reached before the

second week. One should note the higher blood plasma levels of vitamin A and carotene found for Group I from the third to the sixteenth week as compared with those for Group II.

At 4 weeks of age, when the lowest blood plasma vitamin A and carotene values are observed, a significant difference between groups was found. At this age the residual effects of colostrum feeding for the first 3 days are at a minimum, and the low roughage consumption does not greatly

TABLE 2
Sex distribution and average weight gains of calves in Groups I and II

Age of calf	Group I Extra colostrum				Group II No extra colostrum			
	No. & sex of animals		Av. weekly gain per calf	Av. cumu- lative gain of calves	No. & sex of animals		Av. weekly gain per calf	Av. cumu- lative gain of calves
	M	F			M	F		
(wk.)			(lb.)	(lb.)			(lb.)	(lb.)
1	16	20	3.8	3.8	22	18	2.8	2.8
2	16	20	4.7	8.5	22	18	4.0	6.8
3	16	20	5.7	14.2	22	18	5.3	12.1
4	16	20	5.7	19.9	22	18	5.9	18.0
5	13	20	7.8	27.7	20	18	7.9	25.9
6	12	20	8.2	35.9	17	18	8.4	34.3
7	12	20	8.8	44.7	16	18	8.0	42.3
8	10	20	9.1	53.8	13	18	9.7	52.0
9	8	20	13.0	66.8	12	18	11.5	63.5
10	8	20	11.3	78.1	11	18	10.9	74.4
11	8	20	12.3	90.4	11	18	12.3	86.7
12	8	20	13.3	103.7	10	18	13.0	99.7
13	8	20	13.3	116.0	9	18	13.8	113.5
14	8	20	12.5	128.5	8	18	11.8	125.3
15	8	20	14.8	143.3	8	18	12.8	138.1
16	7	20	13.5	156.8	6	18	13.1	151.2
17	7	20	15.7	172.5	5	18	15.0	166.2
18	7	19	15.9	188.4	5	18	13.9	180.1
19	7	19	16.0	204.4	5	18	13.1	193.2
20	5	18	13.0	217.4	5	17	13.8	207.0
21	5	18	17.9	235.3	4	17	13.0	221.0
22	5	18	15.0	250.3	3	17	18.8	239.8
23	4	18	12.3	262.6	3	17	10.9	250.7
24	4	18	15.0	277.6	3	17	14.0	264.7

influence blood plasma carotene and vitamin A values. This significantly higher level of vitamin A must be attributed to the colostrum feeding. The higher blood plasma carotene noted after the eighth week can be attributed to the hay consumption.

It is to be noted that a breed difference occurs at birth in the blood plasma levels of vitamin A and carotene. The blood plasma of the Guernsey calves in this study was significantly lower in vitamin A and higher in carotene at birth than that of the other breeds. No significant seasonal variation in the blood plasma vitamin A was found.

TABLE 3
Blood plasma vitamin A levels of calves used in the experiment
(Vitamin A expressed as γ per 100 ml.)

Group	Breed	Age in days				Age in weeks																	
		1		3		1		2		3		4		8		12		16		20		24	
		No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.	No.	γ/ml.
I	Ayrshire	7	5.6	7	17.5	7	15.7	6	14.6	5	16.2	5	12.7	5	14.6	4	18.3	4	18.4	3	19.1	3	21.4
	Guernsey	7	3.2	7	16.9	8	14.7	8	11.7	8	10.2	8	9.9	7	10.2	7	14.0	7	18.8	6	19.8	6	27.9
	Holstein	8	5.0	8	14.2	8	13.5	8	14.0	8	12.6	8	13.1	8	13.5	7	14.8	7	15.3	6	16.1	5	18.5
	Jersey	6	5.0	6	15.2	6	13.6	6	14.6	6	14.3	6	10.4	4	12.2	5	17.6	5	19.8	5	20.7	5	24.5
	Brown Swiss	5	5.4	5	17.9	5	14.7	5	17.4	5	16.1	5	13.2	5	12.2	5	16.3	5	16.4	3	21.2	3	21.9
	Av.	33	4.8	33	16.2	34	14.5	33	14.7	32	13.4	32	11.8	29	11.8	28	15.6	28	17.3	23	19.1	22	23.2
II	Ayrshire	8	5.8	10	20.4	11	16.1	11	15.7	10	11.9	11	11.9	8	12.3	7	15.6	4	19.9	5	23.2	4	26.1
	Guernsey	9	3.3	9	14.7	9	13.1	9	12.3	9	10.1	9	9.0	7	9.7	7	12.9	6	14.6	5	23.6	5	23.7
	Holstein	6	4.9	6	14.9	7	12.6	7	11.2	7	11.8	7	12.7	7	12.6	5	13.5	5	18.1	5	17.4	4	20.4
	Jersey	8	5.1	7	13.1	9	11.4	9	12.6	9	12.3	9	9.7	8	10.1	7	13.9	5	20.0	4	25.2	4	23.3
	Brown Swiss	3	6.6	4	15.7	4	14.9	4	14.6	4	14.5	3	11.2	4	11.5	4	13.1	4	14.5	3	14.5	3	20.9
	Av.	34	4.9	36	16.1	40	13.5	40	13.3	39	11.8	39	10.8	34	11.2	30	13.9	24	17.3	22	21.2	20	23.1

TABLE 4
Blood plasma carotene levels of calves used in the experiment
(Carotene expressed as γ per 100 ml.)

Group	Breed	Age in days						Age in weeks																	
		1		3		1		2		3		4		8		12		16		20		24			
		No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.		
I	Ayrshire	7	2.2	7	21.8	7	18.2	6	31.2	5	26.7	5	22.5	5	46.7	4	160.6	4	112.6	3	124.0	3	138.5		
	Guernsey	8	5.0	7	65.1	8	72.2	8	73.2	8	61.0	8	60.0	7	104.2	7	162.4	7	253.4	6	235.6	6	287.5		
	Holstein	8	3.5	8	25.4	8	32.2	8	40.4	8	30.1	8	29.1	8	32.2	7	55.0	7	76.0	6	101.2	5	141.5		
	Jersey	5	3.7	6	32.1	6	33.9	6	41.6	6	40.5	6	35.2	4	41.4	5	122.8	5	212.8	5	200.5	5	171.1		
	Brown Swiss	5	3.1	5	39.7	5	37.4	5	34.3	5	18.7	5	13.3	5	19.1	5	89.7	5	47.1	3	131.9	3	80.1		
	Av.	33	3.6	33	36.4	34	39.7	33	46.0	32	37.4	32	32.6	29	51.1	28	115.3	28	144.8	23	164.9	22	172.1		
II	Ayrshire	9	1.5	10	22.3	11	17.7	11	25.7	10	21.8	11	23.3	8	46.3	7	88.7	4	169.9	5	133.5	4	189.5		
	Guernsey	9	4.8	9	75.2	9	66.9	9	72.4	9	60.6	9	65.7	7	109.1	7	152.8	6	213.8	5	267.8	5	337.1		
	Holstein	6	2.3	6	25.6	7	28.4	7	29.9	7	22.6	7	18.8	7	39.5	5	54.4	5	86.8	5	84.7	4	145.5		
	Jersey	8	4.0	7	38.1	9	34.7	9	26.1	9	26.9	9	17.7	8	70.8	7	193.1	4	194.9	4	318.0	4	267.5		
	Brown Swiss	3	2.3	4	44.4	4	34.0	4	21.1	4	15.4	3	12.0	4	17.8	4	45.4	4	60.8	3	128.2	3	114.5		
	Av.	35	3.1	36	41.6	40	36.1	40	36.5	39	31.4	39	30.8	34	60.3	30	119.7	23	148.6	22	135.6	20	234.5		

TABLE 5
The effect of the amount of extra colostrum fed on the blood plasma carotene
and vitamin A and on the cumulative weight gains

Amount of colostrum fed	Day			Week								Cumulated wt. gains
	1	3		1	2	3	4	8	12	16	20	
Over 200 lb. Less than 200 lb. No extra colostrum	3.6	18.0	15.7	13.8	13.3	13.2	13.8	15.2	16.5	20.0	28.0	281.1
	5.0	15.8	14.2	14.3	13.5	11.8	12.6	16.1	17.5	18.7	21.1	275.4
	4.9	16.1	13.5	13.3	11.8	10.8	11.2	13.9	17.3	21.2	23.1	264.7
Over 200 lb. Less than 200 lb. No extra colostrum	4.5	25.4	45.2	55.9	54.5	40.5	54.2	110.4	254.0	196.4	207.2	
	3.2	38.9	38.4	43.4	32.7	30.4	50.1	116.9	108.5	151.1	156.4	
	3.1	41.6	36.1	36.5	31.4	30.8	60.3	119.7	148.6	185.4	224.2	

Table 5 presents data showing the effects of feeding different amounts of colostrum on the blood plasma vitamin A, carotene, and cumulative weight gains of calves. It will be noted that the calves receiving more than 200 lb. of colostrum maintained higher levels of vitamin A in the blood plasma and made greater cumulative weight gains than those receiving less than 200 lb.

DISCUSSION

Considerable research effort has been devoted to the development of calf rations, especially those limiting whole milk consumption. The past world war, with huge demands for food, pressed this development. Research workers (3, 9) have developed limited milk feeding schedules using rations fortified with known essential vitamins. In these experiments colostrum was fed only during the first 3 days and no attempts were made to utilize further the surplus colostrum produced. Wise and LaMaster (19) suggested the use of colostrum and reconstituted skim milk in the feeding of young calves. However, they questioned the advisability of feeding colostrum to older calves.

Responses to colostrum feeding, reflected by the blood plasma levels of vitamin A and carotene, substantiate earlier reports (10, 14). In the experiment herein reported, calves fed surplus colostrum, whenever available, maintained higher blood plasma levels for vitamin A and carotene, made more rapid weight gains and exhibited a more healthy appearance than did the control calves. These calves showed more luster to the hair coat and were more active and alert, especially during the first 2 months. Feeding of surplus colostrum did not prevent cases of scours. Such cases occurred in both groups and resulted in marked drops in the blood plasma vitamin A but did not materially affect the results. As in the previous experiment (14), no cases of scours could be attributed directly to colostrum feeding, even though on many occasions rations were changed abruptly from complete milk rations to complete colostrum rations.

The economic importance of utilizing all colostrum in raising dairy calves is emphasized by this experiment. On the average, with five breeds and all ages of cows represented, the surplus colostrum produced amounted to 51.3 lb. per cow. In this experiment, with 78 cows represented, a total of 4,007 lb. of surplus colostrum was utilized in raising calves. If one-half as much surplus colostrum per cow from each of the 26 million dairy cows in the U.S.A. was utilized in calf raising, it would represent a saving of more than 650 million lb. of marketable milk. Allen (1) earlier recommended use of stored colostrum to replace marketable milk for raising dairy calves. The results of this experiment indicate that colostrum can be used to greatest advantage during the first month of life, when a calf must have milk in some form in its ration. During this period the calf needs special care and a well balanced ration if it is to survive and make thrifty

growth. After 4 to 6 weeks of age the feeding of extra colostrum has less marked effect because of greater consumption of hay and dry concentrate feeds. The consumption of these feeds, no doubt, also affects the rate of microbiological synthesis in the rumen.

SUMMARY

Seventy-six calves, born in The Ohio State University dairy herd, were divided into comparable groups at birth. For the first 3 days all calves received colostrum from their dams. After 3 days, the calves in both groups were fed and managed similarly, except that for calves in Group I, colostrum, whenever available, replaced part or all of the regular ration of Holstein milk. The amounts of either milk or colostrum fed were determined by the body weights of the calves.

Calves in Group I maintained higher levels of blood plasma vitamin A and carotene, made more rapid weight gains, especially during the first 6 weeks, and exhibited a superior physical appearance. Abrupt changes in the amounts of colostrum fed, which in some instances varied from no colostrum to all colostrum, did not create any special management problems. The calves did not scour from colostrum feeding.

Complete utilization of all colostrum for calf feeding is important from an economic standpoint. Only 30 per cent of the colostrum produced in The Ohio State University dairy herd during the calendar year was used in feeding calves during the first 3 days. The balance, which exceeded 4,000 lb., was used to replace an equal amount of marketable milk in the feeding of calves in Group I. The general practice of using all the colostrum produced in the raising of calves would result in a substantial saving of marketable milk.

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CAROTENE REQUIREMENTS FOR GUERNSEY AND JERSEY CALVES AS DETERMINED BY SPINAL FLUID PRESSURE

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Where calves of the various breeds are kept side by side, as in college herds, it has been noted that Guernsey and Jersey calves sometimes are more difficult to rear than Holstein or Ayrshire calves. The view has been expressed that this difference might be due to variations in carotene metabolism.

Boyer *et al.* (1) found that Guernsey calves required 57 γ of carotene per lb. of body weight to maintain a normal vitamin A level in the blood plasma, whereas Holstein calves required only 34 γ per lb. However, Nelson *et al.* (6) and Moore and Berry (4) did not note any difference in plasma vitamin A values from birth to 4 months of age between calves of the various dairy breeds, even when similar conditions of management were followed for all breeds. In these studies vitamin A as present in Holstein milk was fed to all the calves up to 2 months of age.

In order to gain further information on the question of whether there is a difference in the carotene requirements of the various breeds, the writers determined the carotene requirements of Guernsey and Jersey calves during the winter months, using cerebrospinal fluid pressure measurements as a criterion of adequacy.

The normal spinal fluid pressure varies between 75 to 120 mm. of water. Whenever the spinal fluid pressure exceeds 120 mm., it is considered abnormal and is taken as evidence that the calf is deficient in vitamin A. Previously published data (5) have shown that the spinal fluid pressure technique is quite precise and that it is possible to distinguish between differences in carotene intake of amounts as small as 2 γ per lb. of body weight. Where blood data are used, intakes must differ by as much as 10 to 15 γ per lb. before significant differences in blood values occur (5). In work on a considerable number of Holstein and Ayrshire calves, the authors never have observed an increase in spinal fluid pressure when 30 γ carotene per lb. were fed, whereas most calves on 28 γ show an elevated pressure. An increase above normal rather than the absolute value of the spinal fluid pressure is the deciding point in determining whether carotene intake is adequate.

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¹ The data for the Guernsey calves were collected while the senior author was located at the University of Maryland.

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EXPERIMENTAL PROCEDURE

Four Guernsey male calves and four Jersey male calves were used in this experiment to determine the minimum amount of carotene necessary to prevent an increase in spinal fluid pressure. The four Guernsey calves were fed carotene as present in dehydrated alfalfa-leaf meal at the rate of 28, 32, 34, and 36 γ per lb. of body weight, respectively, while the four Jersey calves were fed at the rate of 28, 30, 32, and 34 γ , respectively.

These levels of intake were maintained throughout the winter months, and with one exception all calves were placed on their respective levels of intake during the early fall or winter months. There are some differences in ages of the calves used on the experiment. Previous experience has not demonstrated any marked differences in requirements of calves varying from 4 to 14 months of age. Methods and procedures were the same as previously outlined (5).

RESULTS AND DISCUSSION

Table 1 shows the results for the Guernsey calves. The spinal fluid

TABLE 1
Results obtained with four Guernsey male calves

Date	Age	Weight	Plasma vitamin A	Plasma carotene	Spinal fluid pressure
	(days)	(lb.)	(γ /100 ml.)	(γ /100 ml.)	(mm. H ₂ O)
<i>No. 472 (Intake 28 γ carotene/lb.)</i>					
6-28-42	120	206	5.7	24	
7-28-42	150	225	12.8	38	115
8-27-42	180	262	9.5	48	75
9-26-42	210	281	6.7	34	120
10-26-42	240	296	10.1	32	
11-25-42	270	338	10.1	36	160
12-25-42	300	364	8.6	33	190
1-24-43	330	409	7.3	43	160
<i>No. 496 (Intake 32 γ carotene/lb.)</i>					
11-3-43	240	357	8.8	44	
12-3-43	270	375	11.4	46	90
1-2-44	300	414	11.3	68	230
<i>No. 496 (Intake increased to 40 γ carotene/lb.)</i>					
2-1-44	330	456	14.1	71	160
3-2-44	360	487	12.8	67	90
4-1-44	390	542	...		90
<i>No. 89 (Intake 34 γ carotene/lb.)</i>					
11-15-44	120		9.1	19	
12-15-44	150	182	13.8	39	90
1-14-45	180	209	10.9	54	100
2-13-45	210	236	10.9	67	100
<i>No. 90 (Intake 36 γ carotene/lb.)</i>					
12-15-44	120	181	15.0	29	90
1-14-45	150	206	12.5	49	100
2-13-45	180	241	10.4	45	90

pressure remained within normal limits throughout the experimental period for the two calves (nos. 89 and 90) that received 34 and 36 γ of carotene per lb. daily. At intakes of 28 and 32 γ (nos. 472 and 496) the spinal fluid pressure increased. When the carotene intake of no. 496 was increased to 40 γ per lb. after an increase in spinal fluid pressure had occurred, the spinal pressure values returned to normal in about 60 days. This effect may be partially seasonal, since unpublished data indicate that the cerebrospinal fluid pressure will start to decrease in March due to seasonal differences in carotene requirements. These data indicate that the minimum requirement for carotene by Guernsey calves is near 34 γ per lb. of body weight. The blood vitamin A values shown in this table also are of some interest. The vitamin A values for calf no. 496 during the period when his requirements were not being met (Nov. 3 to Jan. 2) were not noticeably lower than those for no. 89 or 90, and only in calf no. 472 were the vitamin A values below normal more or less consistently.

It will be noted that the spinal fluid pressure of calf no. 472 on the 28 γ level did not increase until he had been on experiment for about 4 months, whereas other calves showed increases in about 2 months. This calf was started during early summer and the delayed response is due to the fact that summer requirements (unpublished data) appear to be less than for the winter months.

Table 2 shows the results for the four Jersey calves. At levels of 32 and 34 γ of carotene per lb. of body weight, the spinal fluid pressure of calves no. 509 and 512 remained within normal limits (75 to 105 mm. H_2O). When the carotene intake of calves no. 2391 and 2392 was maintained at 28 and 30 γ , respectively, the spinal fluid pressure was above normal in both cases. Thirty-two micrograms of carotene per lb. of body weight therefore would appear to be the minimum requirement for Jersey calves, as compared with 34 γ per lb. for Guernsey calves. An examination of the plasma vitamin A values of these Jersey calves shows that these values were generally greater throughout the experimental period when carotene was fed at the highest level, but the differences in vitamin A values between calves no. 512 and 509, no. 509 and 2392, and no. 2392 and 2391 are so small that it is difficult to decide, on the basis of blood data alone, when the requirements actually were being met.

The requirement of 34 γ for Guernsey calves and 32 γ for Jersey calves is somewhat above the 30 γ level previously reported as being the minimum requirement for Holstein and Ayrshire calves. The differences in carotene requirement between the breeds therefore are not marked. In previous experiments with Holstein and Ayrshire calves there never has been an increase in spinal fluid pressure in calves fed as much as 30 γ or more of carotene per lb. of body weight. Yet in these experiments with Jersey and Guernsey calves, two receiving 30 and 32 γ showed elevated pressures. How-

ever, the 34 γ figure for Guernseys is much lower than that reported by Boyer *et al.* (1), whose data indicated a requirement of 57 γ of carotene per lb. of body weight for Guernsey calves and 34 for Holstein calves.

There are three possible explanations for this discrepancy. Boyer *et al.* (1) utilized blood plasma values as a criterion of adequacy. A study of the numerous data collected from this laboratory has shown that there

TABLE 2
Results obtained with four Jersey male calves

Date	Age	Weight	Plasma vitamin A	Plasma carotene	Spinal fluid pressure
	(days)	(lb.)	(γ /100 ml.)	(γ /100 ml.)	(mm. H ₂ O)
<i>No. 2391 (Intake 28 γ carotene/lb.)</i>					
8-29-46	200	285	5.7	39	
9-28-46	230	339	5.5	56	95
10-28-46	260	364	6.5	55	170
11-27-46	290	415	7.8	58	165
12-27-46	320	448	7.9	89	185
1-26-47	350	465	9.7	102	175
<i>No. 2392 (Intake 30 γ carotene/lb.)</i>					
8-31-46	200	231	4.5	68	
9-30-46	230	247	6.8	94	135
10-30-46	260	294	7.8	106	160
11-29-46	290	321	9.0	99	195
12-29-46	320	342	9.1	106	165
1-28-47	350	396	8.6	110	210
<i>No. 509 (Intake 32 γ carotene/lb.)</i>					
8-27-46	190	247	6.4	83	
9-26-46	220	275	6.2	107	75
10-26-46	250	321	8.1	134	80
11-25-46	280	402	9.2	138	75
12-25-46	310	431	11.9	175	75
1-24-47	340	440	10.0	146	80
<i>No. 512 (Intake 34 γ carotene/lb.)</i>					
8-31-46	170	173	7.2	42	
9-30-46	200	213	8.0	51	100
10-30-46	230	237	8.8	77	90
11-29-46	260	300	10.6	62	
12-29-46	290	327	10.8	110	105
1-28-47	320	378	12.7	103	85

must be a spread in intake of at least 10 to 15 γ of carotene per lb. of body weight before a good correlation with blood data can be noted. The blood data in tables 1 and 2 further emphasize this point. Calves on the same intake show considerable individual variation in plasma vitamin A values. There also may be differences in requirements between various strains or families of Guernsey cattle. If inheritance plays some part in causing higher carotene requirements for Guernsey than for Holstein

calves, it is probable that there would be differences in families or strains of Guernseys. This point should be investigated. Another possible cause for the difference between the results from this laboratory and those from Wisconsin might be the difference in some environmental factor. Unpublished data show a higher requirement for the winter months than for the summer months.

The vitamin A content of the blood plasma of the Guernsey and Jersey calves used in this experiment is of the same order as that of the Holstein and Ayrshire calves that were fed at similar levels of carotene intake in previous experiments (5). This would indicate further that the carotene requirement of the Guernsey and Jersey calves used in this experiment was not greatly different from that of the Holstein and Ayrshire calves. Even though the difference is not great, it might account for some of the difficulty that sometimes is encountered in raising Guernsey calves, particularly if the hay quality is very poor or hay consumption is not adequate.

Requirements reported in this experiment are about double those reported by Moore (3) and Guilbert *et al.* (2), where night blindness was used as a criterion. The latter authors term their requirements "physiological minimum". In the light of the present and previous data (5), such an interpretation no longer is tenable. Furthermore, minimum requirements should be based on the least determinable change produced in the animal by a deficiency of the nutrient under study. The carotene requirements for calves as determined by cerebrospinal fluid pressure would appear to meet this definition more closely than night blindness, since changes in spinal fluid pressure occur before night blindness can be detected. The requirements as determined by the night blindness technique therefore cannot be considered adequate.

SUMMARY

1. Guernsey calves under our experimental conditions required an intake of 34 γ of carotene per pound of body weight during the winter months to maintain a normal spinal fluid pressure. On the same basis, Jersey calves required an intake of 32 γ . If inheritance is found to affect carotene requirements, it is possible that these values might need to be modified even for strains within the breed.

2. This requirement is slightly higher than for Holstein and Ayrshire calves, which, under similar conditions, require 30 γ of carotene per lb. of body weight.

3. These results appear to indicate that somewhat more attention should be given to the quality of hay used in feeding Guernsey and Jersey calves than in feeding Holstein or Ayrshire calves.

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MANUFACTURE OF POWDERED CREAM FOR WHIPPING BY AERATION¹

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A number of contributions were made during the war to the knowledge of methods for the successful manufacture of powdered whole milk and ice cream mix. Because of the success of industry with these two items, it is logical to consider the application of dehydration to certain other types of dairy products, such as cream. In 1922 Babcock (2) demonstrated that reconstituted powdered cream containing as much as 40 per cent fat failed to whip. He concluded that such a product may be considered as useless for whipping purposes.

In 1937 Getz *et al.* (3) reported on a method for whipping cream by aeration. The cream is charged with nitrous oxide at a pressure of about 175 lb. in a specially constructed steel container. The cream under pressure is released to the atmosphere through a Schrader valve,² resulting in a greatly expanded volume. While this method involves gasification of the serum portion of the cream, air incorporation in ordinary cream whipping is dependent upon a partial clumping of the fat. Because of this fundamental difference, it was thought that the process of drying cream to be whipped by aeration would have little or no effect upon either the gas dispersion or the stiffness of the whip. This study was made to test this hypothesis.

EXPERIMENTAL PROCEDURE

The cream-mix was made from fresh sweet cream, condensed skim milk, skim milk, sugar, stabilizer and flavoring. It then was pasteurized at temperatures not over 160° F. for 30 minutes and spray dried. Attempts were made to keep the iron and copper content at a minimum. The importance of each of the following factors was studied: (a) composition of the cream-mix; (b) nozzle size; (c) spray pressure; and (d) relation of inert gas and certain antioxidants to keeping quality.

Some of the experiments reported herein were conducted during World War II with a product containing 19 per cent butterfat on a reconstituted basis. Unless otherwise stated, the remainder of the experiments have been conducted with mix containing approximately 30 per cent butterfat on a

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¹ Process and container patented by Aeration Processes, Inc., Columbus, Ohio (U. S. Patent nos. 2,294,172 and 2,281,604).

² The Schrader valve originally employed has been superseded by an entirely new valve of original design (Model F2). This newly constructed valve meets the specifications of the U. S. Public Health Service.

reconstituted basis. Sucrose was used as the sweetening agent and was added before drying. Pure vanilla concentrate was used for flavoring the cream. The cream-mix was not condensed before drying, as the total solids content made this procedure unnecessary. All the cream-mixes were dried on an experimental spray drier using a no. 72 nozzle with a no. 20 core unless otherwise stated.

In the keeping quality studies, the powdered cream-mix was uniformly mixed and packed in no. 1 tin cans, the same weight of dried cream-mix being put into each can. After sealing, the cans were gas packed where necessary for the experiment.

For whipping purposes the dried cream-mix was reconstituted in the proper ratio with cold tap water and held for 24 hours or more at 40° F. Eight ounces of cream-mix were placed in Instantwhip³ containers, which then were charged with nitrous oxide gas and chilled before dispensing. The dispensed product usually was examined for drainage, overrun, flavor, body and appearance. Drainage was measured by dispensing an Instantwhip container into a 6-inch funnel, fitted with a screen at the apex, and set in a graduated cylinder (fig. 1). The volume (ml.) of drainage secured in 30 minutes at room temperature was recorded as the measurement of the amount of drainage.

On the powdered samples held in storage, changes in oxygen concentration in the headspace gas and changes in palatability were obtained at various intervals. The oxygen values were secured by the Manometric procedure of Van Slyke and Sendroy (12) and the flavor scores by two or more judges using the ice cream score card based on a flavor score of 45 out of a possible 50 points.

Moisture was determined by the toluol distillation method (1), butterfat of the powdered cream-mix by the Mojonnier method (8), copper by the procedure of Hetrick and Tracy (5), and iron by the method of Pyenson and Tracy (9).

RESULTS

Effect of variations of butterfat on whipping properties and appearance of reconstituted powdered cream-mix. Cream-mixes containing 26, 30, 32 and 34 per cent butterfat were prepared (batches no. 1, 2, 3 and 4, respectively). The milk solids-not-fat were standardized to 7.5 per cent. Other ingredients added were 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. After drying, a portion of each of the four lots was reconstituted with the proper amount of water, aged for 24 hours, and then placed in the containers, gassed and chilled before dispensing. The samples were shaken uniformly and dispensed simultaneously. There was only 1.5 ml. drainage from the 26 per cent product, 0.5 ml. drainage

³ "Instantwhip" is the trade name for the product as distributed by Aeration Processes, Inc., Columbus, Ohio.

from the 30 and 32 per cent products and no drainage from the 34 per cent product. The flavors of all the samples were identical. As the fat content was increased from 26 to 34 per cent, an improvement in the body and texture resulted and the product became drier in appearance. Since only a slight advantage resulted from using 32 or 34 per cent fat cream-mix as compared with the 30 per cent fat cream-mix, it was decided that future trials with heavy cream-mix would be limited to mixes containing 30 per cent butterfat.

Another factor that influenced the decision to standardize on 30 per cent butterfat was the heavy viscosity obtained with the higher-testing samples. Reconstituted cream-mix made from dried cream has a greater viscosity than the original cream-mix before drying. Too great a viscosity interferes with filling and the containers must be shaken longer to dissolve the nitrous oxide gas in the cream.

Effect of variations in the milk solids-not-fat on whipping properties and appearance of reconstituted powdered cream-mix. The mixes for this study were standardized to contain 30 per cent butterfat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. The variations in the milk solids-not-fat were 6, 7, 8 and 9 per cent (batches no. 5, 6, 7 and 8, respectively). Concentrated skim milk was used to increase the percentage of non-fat milk solids.

On reconstitution, the sample containing the 9 per cent milk solids-not-fat had the greatest viscosity, while the sample with the normal milk solids-not-fat (6 per cent) had the least viscosity. Increasing the milk solids-not-fat produced a heavier body in the whipped cream-mix. The flavor and standing-up qualities of the whipped cream-mix also were improved by the additional milk solids-not-fat.

Drainage from the whipped cream-mix was reduced by increasing the milk solids-not-fat content of the cream-mix. However, 9 per cent milk solids-not-fat in the 30 per cent butterfat cream-mix reduced overrun and produced a moist body. Since too great a viscosity produces mechanical difficulties in filling the containers and since too moist an appearance is produced by more than approximately 8 per cent milk solids-not-fat, it was deemed advisable to limit the milk solids-not-fat content to approximately 8 per cent in 30 per cent butterfat cream-mix.

Comparison of whipped reconstituted powdered cream-mix made with and without added emulsifying agent. These comparisons were made using several emulsifiers. Typical results of a representative emulsifying agent are presented. Cream-mixes containing 29.5 per cent butterfat, 7.5 per cent milk solids-not-fat, and 5 per cent sugar were prepared without and with 0.2 per cent glycerol monostearate. These mixes (batches 9 and 10) were dried and reconstituted in the usual manner. There was no drainage at room temperature in either sample in 0.5 hour. The flavors of both

samples were identical. The sample containing the emulsifying agent whipped to a higher overrun at any given pressure and the whipped cream had a drier appearance, a better body and a finer texture than the control sample containing no emulsifier. Commercial products containing emulsifying agents such as sorbitan monostearate also were found to be satisfactory. Gelatin and sodium alginate stabilizers alone were not satisfactory for powdered cream-mix for whipping purposes. They produced a moist appearance in the whipped cream.

Effect of nozzle size on whipping properties of reconstituted cream-mixes. A mix containing 29 per cent butterfat, 7 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent was used. The nozzles used were nos. 79, 72 and 65, representing openings of 0.0145, 0.025 and 0.035 inch, respectively (batches 11, 12 and 13).

There were no significant differences in whipping properties, body and texture or appearance of whipped reconstituted cream-mixes sprayed with the different nozzles. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with relatively large orifices.

Relation of spray pressure to the whipping properties of reconstituted powdered cream-mix. Homogenization is detrimental to the body, texture, appearance and drainage of cream whipped by aeration. The lower the fat content of the cream, the more detrimental is the effect of homogenization (10).

To determine whether the spraying process has any detrimental effect on the whipping properties of reconstituted powdered cream-mix, a lot of cream-mix (19 per cent butterfat, 9 per cent milk solids-not-fat, 6 per cent sugar, 0.15 per cent gelatin, and 0.2 per cent emulsifying agent) was divided into three portions and sprayed at: (a) 200 lb. pressure, (b) 1,500 lb. pressure, and (c) 2,800 lb. pressure (batches 14, 15 and 16).

The amount of spray pressure used had no significant effect on the overrun obtained on powdered cream-mix whipped by aeration (table 1). The drainage, however, was nearly doubled as the pressure was increased from 200 to 2,800 lb. As the spray pressure was increased, the whipped cream-mix became more moist in appearance and contained larger gas cells. Similar results were obtained with 30 per cent reconstituted powdered cream-mix, except that the detrimental effect of the higher spray pressures was not as pronounced as with 19 per cent cream-mix.

Relation of inert gas and certain antioxidants to keeping quality. In the commercial manufacture of powdered whole milk, replacing air in the package with nitrogen and/or carbon dioxide has been found to decrease the intensity of the oxidized flavor over a period of time and, in some cases, delay onset of oxidized flavor development. In preliminary trials it was shown that dried cream-mix packed in air could be held only a few weeks

at room temperature before a stale or oxidized flavor developed. To have commercial value, it is necessary that dried cream-mix keep for longer periods of time. That the oxidized flavor can be delayed by the addition of certain antioxidants to milk before drying has been demonstrated by Hollender and Tracy (6), Tracy *et al.* (11), Jack and Henderson (7), Waite (13), and Hetrick and Tracy (5).

To determine whether the use of antioxidants would prolong the keeping quality of dried cream-mix, six 50-lb. lots of cream-mix (29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.2 per cent glycerol monostearate) were dried, containing the following levels of antioxidants. The indicated percentages represent the amounts used as calculated on the basis of the weight of the fat: Batch no. 17, control, no added antioxidants; 18, Viobin antioxidant, 0.1 per cent; 19, nordihydroguaiarctic acid (NDGA), 0.03 per cent; 20, gallic acid, 0.1 per cent; 21, sodium

TABLE 1

Effect of spray pressure on whipping properties of reconstituted powdered cream-mix

Batch no.	Spray pressure	Overrun	Drainage	Firmness	Gas cell structure	Dryness
	(lb.)	(%)	(ml.)			
14	200	500	22	Good	Small uniform gas cells	Dry
15	1,500	490	39	Poor	Large irregular gas cells	Moist
16	2,800	510	43	Very poor	Large irregular gas cells	Moist

arabo ascorbate, 0.1 per cent; and 22, natural mixed tocopherols, 0.1 per cent.

All of the antioxidants were added directly to the cream-mix at the preheater just before spray drying, except the NDGA, which was dissolved in 15 mm. of butter oil before adding to the preheater. Both air- and nitrogen-packed samples were prepared. The samples were stored at room temperature. Data taken during 6 months of storage are given in table 2.

Oxygen concentration in the headspace gas gradually was lowered and the dried cream became less palatable as the storage period advanced. A stale flavor usually preceded the oxidized flavor in both air and nitrogen packed samples. After 180 days the nitrogen-packed control sample, although slightly oxidized, was still palatable, while the air-packed control sample had a pronounced oxidized flavor.

At the end of 6 months of storage at room temperature, all air-packed batches containing antioxidants were oxidized except batch no. 22, which had become intensely metallic⁴ after 5 weeks of storage. Through the 121-day storage period, the air-packed batches containing antioxidants scored

⁴ Not to be confused with the typical oxidized or tallowy flavor.

consistently higher than the control that contained no antioxidant. The addition of antioxidants to powdered cream-mix, especially when nitrogen packed, increased the keeping quality of the powder. The most effective antioxidants were NDGA, gallic acid and Viobin. The sodium arabo ascorbate delayed onset of the oxidized flavor but produced a cooked or "nutty" flavor in the cream-mix, which persisted throughout the storage period. Samples containing mixed tocopherols developed a very metallic off-flavor after 5 weeks of storage.

TABLE 2

Changes in oxygen concentration in head space gas and palatability of air-packed and gas-packed dried cream containing antioxidants

Batch no.	Antioxidant		Days storage at room temperature					
			7	14	35	58	121	180
17	Control	% oxygen	20.46		20.62	20.14	19.78	18.79
		Flavor	45		41	39	38.5 ^b	37.5
17N ^a	Control	% oxygen	0.85	1.06	0.84	0.68	0.62	0.28
		Flavor	45		42	41 ^b	40.5	40
18	Viobin	% oxygen	20.11		20.56	20.56	19.77	19.22
		Flavor	45		43	41	40	39.5 ^b
18N	Viobin	% oxygen	1.03	0.76	1.11	0.47	0.52	0.51
		Flavor	45		43.5	42	42	42
19	NDGA	% oxygen	20.0		20.12	20.44	20.11	19.84
		Flavor	45		43.5	43	43	41 ^b
19N	NDGA	% oxygen	1.08	1.02	0.95	1.03	0.19	0.33
		Flavor	45		44	43	43	42.5
20	Gallic acid	% oxygen	20.04		20.35	20.53	20.07	18.11
		Flavor	45		44	43	43	40 ^b
20N	Gallic acid	% oxygen	0.92	0.50	0.51	0.54	0.45	0.21
		Flavor	45		44.5	43.5	43.5	42
21	Sodium arabo ascorbate	% oxygen	20.35		20.3	19.91	19.64	18.26
		Flavor	42.5		42.5	42	41	40 ^b
21N	Sodium arabo ascorbate	% oxygen	1.06	0.71	0.76	0.65	0.56	0.00
		Flavor	42.5		42.5	42.5	42.5	42
22	Natural mixed tocopherols	% oxygen	20.39		20.35	20.09	16.29	18.32
		Flavor	41		35 ^c	33 ^c	30 ^c	25 ^c
22N	Natural mixed tocopherols	% oxygen	0.92	0.41	0.89	0.53	0.19	0.27
		Flavor	41		35 ^c	33 ^c	30 ^c	25 ^c

^a N indicates samples were nitrogen packed. Others were air packed.

^b Time when oxidized flavor first was noticed.

^c Metallic—not to be confused with oxidized or tallowy.

Vanilla as an antioxidant. In another study that was repeated three times with practically the same results, powdered cream-mixes containing NDGA and a pure 6-fold vanilla concentrate (made from Bourbon and Mexican beans) were compared as to keeping properties. The cream-mix had a composition of 30 per cent butterfat, 7.35 per cent milk solids-not-fat, 5 per cent sugar, and 0.2 per cent glycerol monostearate. The NDGA was added at two levels, 0.0025 per cent and 0.005 per cent, based on the

TABLE 3
Changes in oxygen concentration in head space gas and palatability of air-packed
and gas-packed dried cream-mix containing antioxidants

Batch no.	Antioxidant	Days storage at room temperature									
		7	30	60	92	120	152	182	213	265	365
23	Control	20.61 41.5	20.05 38.0 ^b	19.40 36.0	19.16 35.0	18.44 33.0	17.14 33.0	17.11 33.0	15.95 31.0	12.45 20.0	2.12 20.0
23N ^a	Control	1.66 43.0	1.63 39.0 ^b	1.11 39.0	1.37 38.0	0.77 41.0	0.30 40.5	0.65 39.5	0.52 38.0	0.89 36.0	0.21 35.0
24	0.0025% ^c NDGA	20.41 43.0	20.19 41.5 ^b	19.74 39.0	19.58 39.0	19.71 39.0	19.07 38.5	17.16 35.0	18.56 33.0	15.76 34.0	12.70 30.0
24N	0.0025% ^c NDGA	1.65 43.0	1.89 42.5	1.35 43.0	1.65 40.0 ^b	1.37 41.0	0.91 41.0	0.47 40.0	1.00 39.0	0.52 38.0	0.32 37.0
25	0.005% ^c NDGA	20.71 44.0	20.34 42.0	20.19 42.0	20.04 42.0	17.98 38.0 ^b	18.61 39.0	17.13 35.0	17.02 34.0	18.02 38.0	7.26 34.0
25N	0.005% ^c NDGA	2.22 44.0	2.22 44.0	1.45 43.0	2.05 42.0	1.11 40.0 ^b	0.72 41.5	1.23 40.5	1.17 39.5	0.49 40.0	0.15 37.0
26	0.1% pure vanilla conc.	21.04 44.0	20.41 44.0	19.96 44.0	19.93 43.0	19.71 42.0	19.13 41.0 ^b	18.69 37.0	18.40 37.0	15.54 39.0	11.58 34.0
26N	0.1% pure vanilla conc.	2.13 44.0	2.40 44.0	2.04 44.0	2.16 43.5	1.88 43.0	1.51 42.5	1.42 41.0 ^b	2.13 41.0	1.11 41.0	1.23 40.0

^a N indicates samples were nitrogen packed. Others were air packed.

^b Time when oxidized flavor first was noticed.

^c Based on per cent of fat.

weight of butterfat. It was added in 15 ml. of butter oil to the 60 per cent cream-mixes just before drying. To another mix, 0.1 per cent of pure vanilla extract was added just before drying. The data obtained during a year's storage at room temperature are given in table 3.

The antioxygenic properties of the vanilla were discovered when it was added to the cream-mix before drying to note whether the vanilla flavor was lost in the drying operation. This vanilla concentrate was added

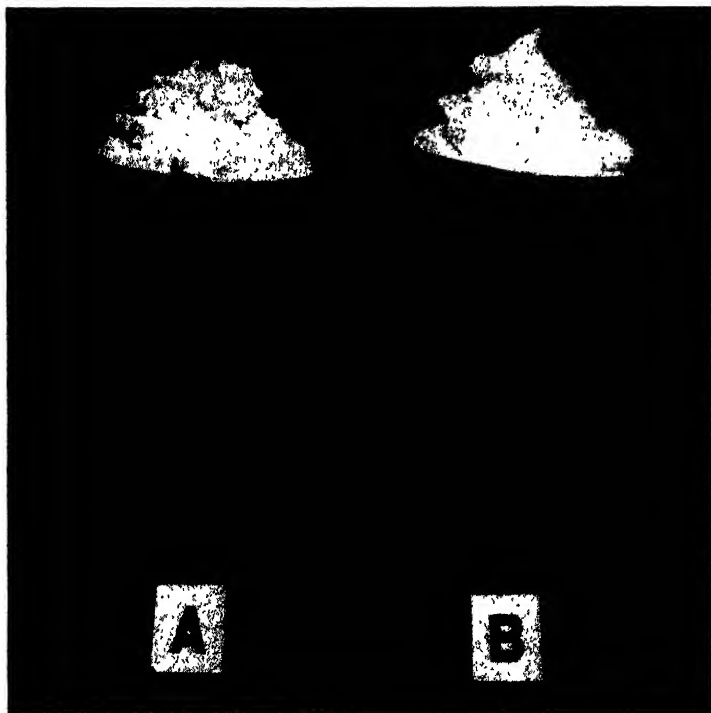


FIG. 1. Method used to measure drainage from whipped cream-mix. (A = Aerated cream made from fresh cream-mix (29.5% butterfat) B = Aerated cream made from reconstituted powdered cream-mix (29.5% butterfat). Both products made from same lot of cream.)

at the preheater just before spray drying the cream-mix at a temperature of 150° F. The processing or the drying operations did not seem to affect the intensity of the vanilla flavor of the reconstituted powdered cream-mix. The data indicate that this pure vanilla concentrate was a more effective antioxidant than NDGA. The nitrogen-packed control sample was oxidized at the 30-day examination period. The sample that contained 0.0025 per cent of NDGA was oxidized at 92 days of storage; when 0.005 per cent NDGA was added, the powdered cream-mix was oxidized at the

120-day examination. Nitrogen-packed powdered cream-mix containing 0.1 per cent of pure vanilla concentrate was not oxidized until examined at 182 days of storage, and then it was only slightly oxidized. Air-packed samples showed similar trends when stored with NDGA, and pure vanilla concentrate. At first the judges thought that the vanilla might be masking the oxidized flavor in the samples containing the vanilla. To check this possible masking, 0.1 per cent pure vanilla concentrate was added to a reconstituted control that was oxidized. The results obtained indicate

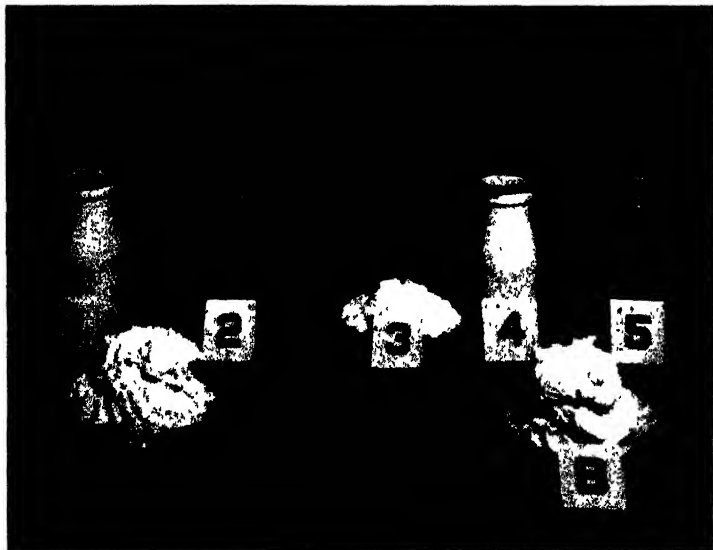


FIG. 2. Comparison of aerated cream made from fresh and reconstituted cream-mix (29.5% butterfat) (1 = Fresh cream-mix; 2 = charged container of fresh cream-mix; 3 = powdered cream-mix; 4 = reconstituted powdered cream-mix; 5 = charged container of reconstituted cream-mix; A = Aerated cream made from fresh cream-mix; B = Aerated cream made from reconstituted cream-mix. Both products made from same lot of cream).

that there was little, if any, masking of the oxidized flavor by the vanilla flavor.

The gas analysis data in table 3 indicate that when the vanilla concentrate was used, in general, there was more oxygen left in the headspace than in the headspace of the control or NDGA samples at the end of the storage period.

The chief constituent of vanilla beans from which vanilla extract is made is vanillin. Vanillin is a phenolic substance having the formula 4-hydroxy 3-methoxy benzaldehyde. Vanillin is the mono-methyl ether of protocatetheric aldehyde, the methoxy group being in the meta position to the aldehyde group. At low concentrations numerous phenolic substances

have the ability markedly to inhibit the autoxidation of fats. The most effective phenols are those which have some type of oxygen linkage in the ortho or para positions, or both, to the hydroxyl group. Some of the best known antioxidants of this type are hydroquinone, the tocopherols, gum guaiac and NDGA. The structural formula of vanillin is quite similar to other compounds that show antioxygenic properties. Consequently, it is possible the antioxidant properties of certain vanillas can be explained through their structural formulas.

Comparison of fresh cream-mix with reconstituted powdered cream-

TABLE 4
Butterfat, moisture, iron and copper content of powdered cream-mixes

Batch no.	Butterfat	Total solids	Moisture	Iron	Copper
	(%)	(%)	(%)	(p.p.m.)	(p.p.m.)
1	66.25	99.5	0.5	4.2	1.18
2	69.06	99.4	0.6	2.4	1.23
3	70.25	99.0	1.0	2.4	0.90
4	72.09	99.6	0.4	2.6	0.78
5	72.04	99.4	0.6	3.8	1.50
6	69.91	99.5	0.5	3.2	1.25
7	69.09	99.4	0.6	3.1	1.43
8	67.03	99.6	0.4	2.5	1.40
9	68.40	99.4	0.6	3.6	0.85
10	68.76	99.3	0.7	3.5	1.23
11	68.20	99.5	0.5	2.2	1.35
12	68.31	99.6	0.4	2.0	1.00
13	68.16	99.5	0.5	1.9	0.95
14	55.19	98.9	1.1	4.2	1.00
15	55.19	98.7	1.3	2.6	1.35
16	55.20	98.9	1.1	2.3	1.20
17	68.42	99.4	0.6	3.1	1.30
18	67.89	99.4	0.6	3.2	1.23
19	67.67	99.2	0.8	2.7	1.18
20	67.79	99.4	0.6	3.7	1.18
21	67.76	99.2	0.8	3.7	1.20
22	67.93	99.2	0.8	2.5	1.15
23	69.92	99.2	0.8	3.0	0.85
24	69.87	99.1	0.9	2.2	0.75
25	69.97	99.4	0.6	1.4	0.63
26	69.89	99.2	0.8	1.8	0.83
27	67.21	98.9	1.1	2.8	1.40

miz. To determine whether or not aerated cream made from powdered cream-mix is as satisfactory as aerated cream made from fresh cream-mix, a batch of cream-mix containing 29.5 per cent fat was divided into two lots. One lot was kept as fresh cream-mix. The other lot was dried in the usual manner and reconstituted with water to bring it back to its original composition of 29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent. Batch no. 27A, made from the fresh cream-mix, is exhibit A in figures 1 and 2 and batch no. 27, made from the reconstituted cream-mix, is exhibit B.

The only differences between the aerated cream-mix and the powdered

cream-mix were the dryness of the whip and the cooked flavor. The body and texture of the fresh product was firm and dry while the reconstituted product was firm but slightly moist. The flavor of the reconstituted product was slightly cooked.

Composition of powdered cream-mixes. Butterfat, moisture and iron and copper content were determined on all of the batches reported in this paper. Table 4 gives a summary of these data. The butterfat percentages of the dried cream-mixes recorded in the table vary somewhat due to variations in composition of the cream-mixes before drying. Batches 14, 15 and 16 were made with 19 per cent cream-mix. All other batches contained 29-30 per cent fat except batches 1, 2, 3 and 4, in which the butterfat was varied from 26 to 34 per cent. No difficulty was encountered in producing a cream-mix with low moisture content, since the dried product consisted of almost 70 per cent butterfat. The iron and copper contents of the dried cream-mixes are similar to the iron and copper contents of powdered whole milk made with the equipment used.

SUMMARY

Studies were made of variations in percentage of butterfat, milk solids-not-fat, and emulsifying agents in powdered cream-mixes. A product consisting of approximately 30 per cent butterfat, 7 to 8 per cent milk solids-not-fat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla concentrate on a reconstituted basis gave satisfactory results. This cream-mix, when whipped by aeration, produced results similar to those obtained with the undried product.

There were no noticeable differences in whipping properties of reconstituted whipped cream-mix made from cream-mix sprayed with nozzle nos. 64, 72 and 79. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with larger orifices.

High spray pressures were found to be detrimental to the whipping properties of the reconstituted cream-mix, producing a product lacking in stability, containing large gas cells and having a moist appearance.

The keeping quality of the powdered cream-mix can be improved by packing in inert gas and by the addition of certain antioxidants before drying. As with powdered milk, it is desirable when storing the powdered cream-mix in inert gas to obtain a low oxygen content of the headspace on desorption. The antioxidants seemed to be more effective when the samples were packed in inert gas than in air. The most effective antioxidant was pure vanilla concentrate. Others that were effective were NDGA, gallic acid and Viobin.

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EFFECT OF DELAY IN DILUTING AND COOLING ON KEEPING QUALITY OF BULL SEMEN

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Technique used in the handling of bull semen and in the breeding of cows artificially has made great progress in the last decade. Even so, many problems still confront workers in artificial breeding. Although improved diluters are now in use and the cooling of semen to between 35 and 40° F. is almost universally practiced, very little experimental work has been done on the time effect of immediate or delayed dilution and cooling. Probably the standard practice is to collect the semen and try to hold it as near the ejaculation temperature as possible until returning to the laboratory, where cooling and diluting procedures are inaugurated. The time required for collecting two ejaculates from a bull and returning to the laboratory, of course, varies according to how far the bulls are from the laboratory and how quickly the ejaculates may be collected from each individual bull. In some cases this time may be negligible, but in other cases enough time undoubtedly is consumed that it may mean the difference between semen that would rate "good" and "poor".

Reports on actual experiments testing the effects of delay in diluting or cooling of semen have not been found. However, procedures described by various workers (2, 3, 5, 6, 7, 8, 11) indicate that diluting and cooling should immediately follow collection and that cooling should be done slowly. One report (4) gives evidence that it is not necessary to cool the semen slowly.

In Louisiana and other southern states the temperature shock to spermatozoa, particularly that due to cold weather, probably is not as important as it is in more northern climates. This study was conducted in an effort to determine if the keeping qualities of bull semen are affected by the time between ejaculation and dilution and the beginning of cooling.

EXPERIMENTAL PROCEDURE

Forty-two ejaculates from six different dairy bulls (five Holsteins and one Jersey) were used for this study. Following collection of each sample, three 1-ml. samples were taken from it for the experiment. These samples were treated as follows: No. 1, the diluting and cooling procedures were started immediately; no. 2, the semen was diluted immediately but the cool-

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ing procedure was not started until 45 minutes following collection; no. 3, both the diluting and the start of the cooling procedure were delayed for 45 minutes from time of collection.

The experiment was conducted between February 18 and April 29, 1947, inclusive. On the 11 actual experimental days during this period the air temperature as reported by the Baton Rouge weather bureau at 10:30 a.m. ranged from 44 to 80° F. and averaged 63.6° F.

The fresh semen was examined microscopically and rated into classes as described by Herman and Swanson (4), except that evaluations were made on the basis of 0.5 intervals. The same system of rating the samples was followed after storage at 35–40° F. for 24 and 72 hours. A variation of the methylene-blue test (1) was run on all samples, initially, and 24 and 72 hours after collection. One milliliter of fresh semen was mixed with 9 ml. of diluter. By using a small test tube, 1 ml. of methylene-blue solution was added to the diluted semen and mixed well. A 0.5-inch layer of mineral oil was added on top of the mixture to seal the tube, which then was placed in a water bath at 40° C. and the reduction time carefully noted. A variation of Beck and Salisbury's test (1), incubating the samples at 115° F. for 15 minutes and examining microscopically, was used on all samples 72 hours after collection.

The first 19 semen samples were diluted with synthetic pabulum (8) and the remaining 23 samples with yolk-citrate diluent (9). In no case were the two diluents used on portions of the same semen sample. All samples were diluted at the rate of 1:10, using 1 ml. of semen to 9 ml. of diluent, which first had been warmed to around 85 to 90° F.

A thermos bottle one-half full of water cooled to 35° F. was used when procedure called for the beginning of cooling immediately after collection. By slipping the vial of diluted semen partially through a hole in the thermos bottle stopper, gradual cooling was begun immediately. After returning to the laboratory, the temperature was taken and the vials of semen placed in 400-ml. beakers one-half full of water at a temperature the same as that of the semen. The beakers were placed in a refrigerator set at 35–40° F. and continued to be cooled until the desired storage temperature of about 38° F. was reached.

Where cooling was to be delayed 45 minutes—*e.g.*, until returning to the laboratory—the semen was diluted in a test tube and placed in 400-ml. beakers one-half full of water at about 85° F. The large beakers containing tubes of semen then were placed in a refrigerator and gradually cooled over a period of 2 to 2.5 hours to temperatures ranging from 35 and 40° F.

In analyzing the results of this study an analysis of variance (10) was run separately on: (a) Motility 24 hours after collection, (b) motility 72 hours after collection, (c) methylene-blue reduction time 24 hours

TABLE 1

*Comparison of motility ratings after storage for 24 and 72 hours between semen samples processed immediately and those delayed in diluting and cooling
(Mean of 42 samples)*

Procedures	Mean motility rating		
	After storage for 24 hr.	After storage for 72 hr.	72 hr. storage and incubation
No. 1, immediate diluting and cooling	3.13	2.20	1.33
No. 2, diluted immediately, cooling delayed 45 min.	3.11	2.04	1.12
No. 3, diluting and cooling delayed 45 min.	2.96	1.81	1.02

after collection, (d) methylene-blue reduction time 72 hours after collection, and (e) incubation test 72 hours after collection.

RESULTS

Motility ratings. Only slight differences in motility ratings (table 1) were noted between the averages for the three procedures based on observations made after 24 hours of storage. The mean value of 3.13 for no. 1 (immediate diluting and cooling) and 3.11 for no. 2 (immediate diluting and delayed cooling) samples indicated little advantage for immediate cooling. A greater effect was evidenced by the delay for 45 minutes in both diluting and cooling (no. 3), in which case the average was 2.96. Differences found after 24 hours of storage were highly significant statistically ($P < 0.001$), as were those found after 72 hours of storage.

Following 72 hours of storage, the average motility ratings (table 1) for the three procedures were 2.20, 2.04 and 1.81, respectively. These differences were greater than those found after 24 hours of storage and indicated that a delay in cooling or in both diluting and cooling tended to lower semen quality. This trend also is shown in the motility averages for

TABLE 2

*Comparison of methylene-blue reduction time of semen samples treated differently following collection
(Mean of 42 samples)*

Procedures	Mean reduction time	
	After storage for 24 hr. (min.)	After storage for 72 hr. (min.)
No. 1, immediate diluting and cooling	15.76	19.21
No. 2, diluted immediately, cooling delayed 45 minutes	16.91	20.14
No. 3, diluting and cooling delayed 45 minutes	17.50	22.88

samples stored for 72 hours and then subjected to incubation at 115° F. for 15 minutes prior to examination. These averaged 1.33, 1.12 and 1.02, respectively, for the three procedures.

Methylene-blue reduction time. As in motility ratings, the time required to reduce methylene blue (table 2) likewise indicated advantages for the immediate dilution and cooling of the semen samples. After storage for 24 hours the reduction time for the three procedures was 15.76, 16.91 and 17.50 minutes and after 72 hours of storage 19.21, 20.14 and 22.88 minutes, respectively. While the trend was the same for the tests made at the two periods of time, only those differences found for the 24-hour period proved to be statistically significant ($P < 0.001$).

SUMMARY

Forty-two ejaculates from 6 different bulls were used in an experiment to determine if the amount of time required to collect semen and get it to the laboratory before dilution and beginning of cooling would affect the keeping qualities of the semen.

Using motility ratings from microscopic examinations made after 24 and 72 hours of storage before and after incubation and methylene-blue reduction tests as criteria of semen quality, it was found that the immediate processing of semen following collection was desirable for the maintenance of desirable characteristics of semen. Delay in either the diluting or the start of the cooling process tended to lower ratings made of the semen.

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EFFECT OF FEEDING TOCOPHEROLS TO DAIRY COWS ON THE QUANTITY AND THE FAT CONTENT OF MILK PRODUCED¹

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Preliminary results obtained in an experiment designed to determine the effect of feeding mixed tocopherols to dairy cows on their milk and butterfat production do not agree with those presented recently by Harris *et al.* (1). These workers reported that the feeding of mixed tocopherols at the rate of 1.0 g. daily per cow brought about an increase of about 27 per cent in the fat concentration and 21 per cent in the total quantity of milk (4 per cent fat-corrected) produced. Their experiment, however, was carried on in a privately owned herd and the milk was tested for butterfat content on only 2 days each month.

EXPERIMENTAL

Seventeen purebred cows, ten Jerseys and seven Holsteins, of various ages were used in the present study. They were divided into two groups with eight cows in the control and nine in the supplemented group. The breed and age of cows used in both groups and their further division into classes A, B and C according to dates of calving are indicated in table 1.

The experiment started with a 10-day preliminary period, which ended May 21, 1947. During this period, none of the cows in either group was fed the tocopherol supplement. Feeding of the supplement, known as "Myvadry", to cows in the supplemented group was started on the evening of May 21 and continued through July 5, 1947. As in the experiment of Harris *et al.* (1), the supplement was added to the grain ration at time of feeding, in such amounts as to provide each cow with 1.0 g. of mixed tocopherols daily.

Cows in both groups were fed and managed alike except for the supplement. All cows were fed normal herd rations, which included good quality alfalfa hay and corn silage, along with concentrates in amounts according to their milk production. The Jerseys were fed grain at the rate of 1 lb. to every 2.5 to 3.5 lb. of milk produced and the Holsteins 1 lb. to every 4 to 5 lb. of milk produced daily. The grain mixture contained 225 lb. ground corn, 300 lb. ground oats, 300 lb. ground barley, 75 lb. linseed meal, 75 lb. soybean oil meal, 10 lb. steamed bone meal, and 15 lb. salt. When weather permitted, cows were kept outdoors except when being fed. Beginning on June 5, all the cows in both groups were turned on pasture

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daily but they continued to receive other roughage and the usual amounts of grain.

Records were kept of the amount and the fat content of milk produced daily by each cow during the entire period of the experiment, including the 7 days after feeding of the supplement had been discontinued. The Babcock method was used in determining the per cent of fat in the milk. Table 2 shows the average per cent of fat in the milk and the calculated average daily production per cow in pounds of 4 per cent fat-corrected milk of cows in each class and group during the preliminary period and at

TABLE 1
Breed, date of birth and date of calving of cows used in the experiment

Animal no.	Breed	Class ^a	Date of birth	Date of calving
Control group				
256	Jersey	A	1-24-39	3-28-47
295	"	A	12-20-42	4- 6-47
302	"	A	2-27-44	3-28-47
275	Jersey	B	7- 2-40	12-26-46
299	"	B	11-10-43	12-23-46
474	Holstein	C	12-23-37	2-20-47
813	"	C	4- 8-41	1-13-47
838	"	C	11-17-43	1-15-47
Supplemented group				
282	Jersey	A	9-20-41	4-12-47
300	"	A	12-18-43	4- 1-47
267	Jersey	B	12-15-39	1-31-47
280	"	B	6-26-41	1-23-47
297	"	B	2-24-43	1- 6-47
480	Holstein	C	3- 2-38	2- 3-47
492	"	C	11-21-39	1- 5-47
814	"	C	5-14-41	12-25-46
835	"	C	5-25-43	2-27-47

^a The calving dates of cows in Classes A, B and C in the control group are approximately the same as of those in the corresponding classes in the supplemented group.

5-day intervals while supplement was fed and also during the 7-day period after feeding of supplement was discontinued.

The condition, general appearance and appetite of all animals remained good throughout the entire period of the experiment, with no class or group showing any superiority in any respect. The data in table 2 fail to indicate any tendency of a rise in the fat content of the milk from the cows in the supplemented group after feeding of the tocopherol was started on the evening of May 21; neither is there any evidence of a drop in the fat percentage during the 7 days of observation after feeding of the supplement was discontinued on July 6. Likewise, the supplementation had no

TABLE 2
Average per cent of fat in milk produced and daily milk production (4% fat-corrected milk) of cows by classes
and by entire groups during the indicated periods^a

Group	Class	May			June							July	
		12-21	22-26	27-31	1-5	6-10	11-15	16-20	21-25	26-30	1-5	6-12	
% of fat in milk													
Control	A	4.73	4.73	4.52	4.60	4.97	5.09	4.75	4.80	4.69	4.60	4.72	
Supplemented	A	4.64	4.37	4.49	4.37	4.74	5.00	4.61	4.61	4.50	4.64	4.48	
Control	B	5.13	5.21	5.32	5.23	5.63	5.58	5.58	5.49	5.18	5.38	5.37	
Supplemented	B	4.88	4.64	4.82	4.81	5.18	5.25	5.07	4.94	4.86	4.96	4.99	
Control	C	3.12	3.07	3.12	3.12	3.13	3.23	3.04	2.96	2.95	3.09	3.04	
Supplemented	C	3.15	3.22	3.18	3.24	3.20	3.26	3.21	3.10	2.92	3.17	2.95	
Control	All	4.14	4.10	4.07	4.09	4.27	4.37	4.13	4.11	4.00	4.09	4.10	
Supplemented	All	3.87	3.86	3.93	3.90	4.10	4.18	4.03	3.93	3.79	3.98	3.84	
lb. 4% fat-corrected milk													
Control	A	31.8	29.8	28.0	26.9	30.8	30.3	28.7	28.7	26.2	24.4	25.1	
Supplemented	A	37.6	36.8	35.9	35.0	38.6	38.7	36.5	34.1	30.5	28.4	28.9	
Control	B	21.4	19.8	20.0	19.7	22.1	22.5	21.2	21.4	18.3	19.9	18.2	
Supplemented	B	21.7	21.4	20.0	20.0	23.4	23.3	22.6	22.4	20.4	20.2	20.4	
Control	C	25.0	24.5	23.5	23.5	26.4	26.6	25.6	24.7	22.8	22.2	21.9	
Supplemented	C	28.6	26.3	24.9	26.5	26.9	28.5	26.6	26.7	23.9	23.3	22.4	
Control	All	26.6	25.3	24.3	23.8	27.0	27.0	25.7	25.4	22.9	22.6	22.3	
Supplemented	All	28.2	26.3	25.7	26.2	28.4	29.0	27.5	26.9	24.2	23.6	23.3	

^a None of the cows in either group was fed the tocopherol supplement during the first and the last periods indicated.

apparent effect on the quantity of milk produced, as expressed in terms of 4 per cent fat-corrected milk. Although the cows in the supplemented group produced at a somewhat higher level than those in the control group, this difference was as great during the preliminary period as it was later during the time when the supplement was fed.

Both the per cent of fat in the milk and the amounts of 4 per cent fat-corrected milk produced by cows in each class and group varied widely from period to period during the progress of the experiment. These variations, however, are no more marked in one class or group than in the other; furthermore, they show a definite tendency to occur simultaneously in the several classes and groups as though resulting from a common cause. Such an effect is indicated by the marked increase in milk production and to a less marked degree by the rise in the per cent of fat in the milk produced by cows in all classes of both groups after they were turned on pasture on June 5.

A more comprehensive study of this problem is under way, the results of which will be presented at some later date.

SUMMARY AND CONCLUSIONS

A feeding trial was conducted to determine the effect of supplementing the rations of lactating dairy cows with 1 g. of mixed tocopherols daily per cow on the amount and fat content of the milk produced. Seventeen cows were used, eight of which received basal ration and the remainder received supplemental tocopherol daily from May 21 to July 5, inclusive. Milk from each cow in both groups was weighed and tested for butterfat content daily.

Neither the amount nor the fat content of the milk produced appeared to be affected by feeding of the tocopherol supplement. Such supplementation produced no changes in the appearances and appetites of the animals.

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THE ELIMINATION OF INTERFERING SUBSTANCES IN THE KAY-GRAHAM PHOSPHATASE TEST WHEN USED FOR HARD RIPENED CHEESE¹

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The Kay-Graham phosphatase test (5), including the Gilreas and Davis modification (4), is not suitable for the analysis of hard ripened cheese. A recent report by Gilreas (3) comparing the effectiveness of several phosphatase tests in the examination of hard cheese emphasized this point when he stated the following concerning the Kay-Graham method: "... the control values particularly in the examination of aged cheese are so high as to limit sharply the utility of this test for detecting the presence of the active enzymes in the sample. This interference is undoubtedly caused by amino acids, particularly tyrosine which is always present in aged cheese." Other phosphatase tests such as those of Sanders and Sager (8) and Scharer (9) would not encounter this type of interference as 2,6-dibromo-quinone-chloroimide (BQC), the color reagent used for these tests, is specific for phenol, whereas the Folin-Ciocalteu color reagent used in the Kay-Graham test originally was developed to determine tyrosine and tryptophane (2).

Results from recent investigations upon the protein decomposition products of cheese (1, 6) encouraged the authors to believe that all the interfering substances could be eliminated in the application of the Kay-Graham test to cheese. It was obvious that tyrosine and possibly tryptophane are interfering substances and that the amine, tyramine, which was shown (6) to be present in all cured commercial Cheddar cheese, also contributed greatly to the interference. This amine is water soluble and forms a blue compound with the Folin-Ciocalteu reagent. In the standard Kay-Graham method it passes into the filtrate to intensify the color.

To eliminate all interfering amino acids and amines from the Kay-Graham test, certain solubility principles were considered. Tyrosine and tryptophane, as well as most of the other amino acids, are insoluble in ether. Tyramine is soluble in ether when extracted under slightly alkaline conditions, but it is insoluble in ether under acid conditions. Phenol, on the other hand, is very soluble in ether under acid conditions.

EXPERIMENTAL RESULTS

Utilizing the solubility characteristics of the various compounds in-

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volved, the following approach, designated as the trichloroacetic acid technic, was evolved.

The method. A sample of ground cheese (0.5 g.) was incubated with 10 ml. of buffer substrate and two drops of chloroform for 24 hours at 37° C. The buffer substrate contained 1.09 g. disodium phenyl phosphate and 17.54 g. of sodium barbital per liter of water, to which 10 ml. of chloroform were added. Following this incubation, 1 ml. of a 25 per cent trichloroacetic acid solution was added. The resulting precipitate was filtered off through Whatman no. 42 filter paper. Five milliliters of the clear filtrate then were pipetted into a standard Mojonnier extraction flask. Enough 1 per cent hydrochloric acid solution was added to this flask to bring the liquid to the bottom of the neck of the flask. Then 25 ml. of ethyl ether were added. The Mojonnier flask was stoppered with a cork covered with tinfoil and inverted slowly 20 times. After the agitation was completed, the clear ether was poured off into a large tube containing 5 ml. of distilled water. The ether was boiled off in about 5 minutes by immersing the tube in a beaker of hot water.

To the 5 ml. of remaining aqueous solution, 2 ml. of Folin-Ciocalteu reagent (diluted 1 to 2) were added, followed immediately by 2 ml. of 14 per cent sodium carbonate. The mixture was placed in boiling water for 5 minutes, after which it was cooled, filtered and the color readings taken. A Luximeter colorimeter was used throughout the entire experimental work.

Phosphatase and interfering blank values of Cheddar cheese. Data were obtained on an assorted group of 25 representative commercial Cheddar cheeses. In table 1, columns 4 and 5, the phosphatase values and interfering blank values of the different cheeses are shown as obtained by the trichloroacetic acid technic. The interfering blanks were obtained by using a plain buffer solution without the disodium phenyl phosphate substrate and by eliminating the incubation period which showed the possibility of removing interfering substances present in the original cheese. Otherwise the method was similar. Controls also were run on many of the cheeses. In column 6, table 1, are shown the interfering blank values obtained on the cheeses by the standard method of Kay-Graham (4, 5) using a 0.5-g. sample of cheese.

The phosphatase values obtained by the trichloroacetic acid technic show that a wide variation existed among these cheeses. Some of these tested cheeses were made from raw milk and some were made from pasteurized milk. The important fact is that all the cheeses tested, ranging in age from 7 months to 10 years, showed interfering blank values which were extremely low, varying from 0.002 to 0.009 mg. phenol per 0.5 g. of cheese and averaging 0.004 mg. per 0.5 g. of cheese. Control values on the cheeses tested also were extremely low. The final filtrates in the trichloroacetic

technic always were clear blue, being very similar in character to those obtained on milk. It was very easy to read intensity differences.

The interfering blank values obtained using the standard Kay-Graham method (table 1), with one exception, were all extremely high, indicating gross contamination by interfering substances produced during ripening.

TABLE 1

The phosphatase and interfering blank values of 25 commercial Cheddar cheeses obtained by the trichloroacetic acid modification of the Kay-Graham method, the Standard Kay-Graham method, and the Sanders-Sager method

Cheese			Trichloroacetic acid technic		Standard Kay-Graham method	Sanders-Sager method ^a
No.	Age	Mfgs. report	Phosphatase values	Interfering blank values ^b	Interfering blank values ^b	Phosphatase values
	(mo.)		(mg. phenol/0.5 g. cheese)		(mg. phenol/0.5 g. cheese)	(γ phenol/0.25 g. cheese)
1	8	Past.	0.013	0.004	0.144	2.5
2	19	Past.	0.010	0.006	0.265	3.0
3	8	Past.	0.014	0.003	0.188	3.0
4	17	Past.	0.012	0.005	0.302	1.0
5	18	Past.	0.013	0.002	0.271	2.0
6	10	Past.	0.004	0.002	0.360	2.0
7	8	Past.	0.020	0.002	0.225	5.0
8	11.5	Past.	0.046	0.004	0.200	6.0
9	8	Past.	0.045	0.005	0.122	6.0
10	13	Past.	0.060	0.004	0.350	9.0
11	14	Raw	0.698	0.003	0.331	35.0
12	126	Raw	0.785	0.007	0.703	> 40.0
13	8.5	Raw	1.226	0.006	0.241	> 40.0
14	16	Raw	0.821	0.004	0.382	> 40.0
15	15	Raw	0.769	0.006	0.422	40.0
16	38	Raw	0.745	0.006	0.466	30.0
17	7	Raw	0.849	0.004	0.181	> 40.0
18	41	Raw	0.702	0.003	0.703	40.0
19	35.5	Raw	0.978	0.006	0.497	> 40.0
20	13.5	Raw	0.773	0.006	0.125	> 40.0
21	8	Raw	0.853	0.002	0.038	> 40.0
22	7	Raw	0.637	0.003	0.166	> 40.0
23	8	Raw	0.853	0.009	0.196	> 40.0
24	29	Raw	0.749	0.009	0.396	35.0
25	16	Raw	0.702	0.004	0.125	> 40.0

^a A value of over 3.0 for the Sanders-Sager method indicates cheese made from improperly pasteurized milk.

^b Shows amount of interfering substances developed in cheese during ripening and not removed by the trichloroacetic acid technic.

The range extended from 0.038 to 0.703 mg. phenol per 0.5 g. cheese. In cheese of low phosphatase values, the interfering blank values often were as much as 60 times as great as the actual phosphatase value of the cheese.

Although it was evident that the interfering substances could be eliminated completely, there was as yet no evidence to show that by using this

technic it would be possible to follow differences in the phosphatase concentration of various cheeses comparable to those shown by standard phosphatase methods. To check this point, all the cheeses were tested by the phosphatase method of Sanders and Sager (8) and these results were compared to results using the new technic, even though there is now no intent of presenting a final procedure for the new technic. The dilution procedure for the Sanders-Sager method which would make the results of those cheeses containing more than 40 γ of phenol per 0.25 g. cheese more quantitative was omitted although this omission does not prevent a correct interpretation of these results. The data, presented in table 1, include the heat treatment history of the cheese milk and the age of the various cheeses, both as given by the manufacturers. It was evident from the results obtained by the Sanders-Sager phosphatase test that some of the cheeses, stated to have been made from pasteurized milk, actually were made from underpasteurized milk. Although at the present time data are not available to show at what concentration of phenol the trichloroacetic acid technic distinguishes raw from pasteurized milk cheese, it is encouraging to note that where there are phenol-value increases in the Sanders-Sager method (table 1), there also are phenol-value increases in the trichloroacetic acid modification (column 4, table 1) of the Kay-Graham method, although not necessarily of the same magnitude. With a few exceptions, the phenol values of the cheese were related to the reported pasteurization of the milk.

The magnitude of the phenol values for cheese obtained by the trichloroacetic acid technic corresponds relatively closely to that obtained on milk with the standard Kay-Graham method, although the critical value dividing the raw from the pasteurized product does not appear to be quite the same.

Interfering substances other than tyrosine and tyramine. It has been reported by Leahy *et al.* (7) that the Folin-Ciocalteu reagent also gave strong colors with a variety of chemical compounds including diacetyl, acetylmethylcarbinol, cystine, l-leucine, indole, uric acid, allantoin and guanine. Although none of these compounds has been reported in cheese in significant amounts, it may be well to point out that most of them are insoluble in ether and others are insoluble in aqueous solutions. However, diacetyl is considered to be soluble in both ether and water. Although only traces have ever been reported in cheese, a study was made to note the effect of the addition of small amounts to cheese. The amounts selected were one to ten times that usually found in ripened butter, which contains on the average of about 3-4 p.p.m. No significant increase was noted in the final readings after these additions, indicating even if diacetyl were ever present it would not affect the final results. The very fact that it was possible to test 25 cheeses of vastly different history and obtain low blanks is another indication that these interfering substances are no longer significant using the modified method.

DISCUSSION

The Kay-Graham phosphatase test as commonly used for milk has been found inaccurate on ripened hard cheeses because of the non-specificity of the Folin-Ciocalteu reagent toward phenol. As the Kay-Graham test is so valuable in the dairy industry, any attempt to overcome its inaccuracy on cheese should be encouraged. Although at this time no attempt has been made to present a routine method for the Kay-Graham phosphatase test on cheese, the removal of the interfering substances should stimulate development of such a method. Additional information is being gathered to perfect the details of the new technic and to show the adaptability and sensitivity of this technic in distinguishing cheeses made from milk heated at different times and temperatures.

The principle employed for the elimination of interfering substances actually consisted of producing conditions which allowed for the selective removal of most of the free phenol produced. The free phenol was produced first in an alkaline medium by the phosphatase enzymes and then extracted with ether under acid conditions so that none of the amino acids or amines likely to produce interference would be extracted. Removal of free phenol by washing with ether is a common practice in the purification of some chemical reagents and biological materials containing phenolic substances. It then was possible to retain the phenol in an aqueous solution by boiling off the ether. Because of its high boiling point (182° C.), probably very little phenol would be lost.

There is no doubt that tyramine is a very important factor contributing to the blue interference, as every cheese in this series contained it in some concentration. If any other products derived from the amino acids or other chemical substances were responsible for interference, they also are ether insoluble, as almost negative interfering blanks and controls were obtained using the trichloroacetic technic.

The use of trichloroacetic acid was found very desirable because it possesses flocculating properties without requiring the aid of heat. It also aided in better color development for some unknown reason. In addition, a clear filtrate always was obtained prior to extraction. However, trichloroacetic acid is soluble in ether and in order to keep the filtrate at pH 1-2 during the extraction, an inorganic acid was added.

Occasionally an emulsion was encountered in the extraction process. This easily was broken by running hot tap water over the lower chamber of the Mojonnier flask for about 20 seconds with the cork loosely stoppered, followed by rapid cooling under the cold water tap.

The standard Kay-Graham method for milk expresses the results in units of *mg. phenol per 0.5 ml. milk* and uses only an aliquot portion of 0.5-ml. milk in the test. In the interests of uniformity the unit used for the trichloroacetic acid modification is *mg. phenol/0.5 g. cheese* even though

here again an aliquot sample smaller than 0.5 g. cheese is used for the final color determinations. For basic quantitative measurement of phenol in certain studies all dilutions should be considered. This can be done easily in this modification, if desired, but in the case of the phosphatase test where the method is mainly empirical in nature this is not considered essential.

SUMMARY

A technic was developed for the selective separation of free phenol from interfering substances in the Kay-Graham phosphatase test when used on hard ripened cheese. Tyrosine and tyramine were important interfering substances.

The elimination of the interfering substances was accomplished by using trichloroacetic acid as a precipitant for the cheese proteins, and by extracting the free phenol, formed as a result of phosphatase activity in an alkaline substrate, with ether under acid conditions using a Mojonnier-type extractor. The ether containing the phenol was placed in distilled water and then was boiled off. The aqueous solution then was treated with the Folin-Ciocalteu reagent and the amount of phenol determined with a colorimeter.

The amount of interfering substances in ripened cheese not removed by using the new trichloroacetic acid technic was extremely low, as values averaging 0.004 mg. phenol per 0.5 g. cheese were obtained for 25 cheeses varying in age from 7 months to 10 years.

Phenol values obtained on cheese using the new technic showed changes in phosphatase activity which corresponded well with results obtained on the same cheeses with the Sanders-Sager phosphatase method. The phenol values obtained using this new technic on cheese were on the order of those obtained on milk using the standard Kay-Graham method, but no complete data have been obtained yet to establish the final details of the test and to show at what point the new technic would distinguish raw from pasteurized milk cheese.

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DEHYDRATED SWEET POTATOES AS A CONCENTRATE FEED FOR DAIRY CATTLE¹

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Selection of the best quality sweet potatoes for table use yields culls which may be dehydrated and used for animal feed. High yields of sweet potatoes in some areas may justify growing them specifically for animal feeding, especially where corn yields are low. The present study was undertaken to determine the value of dehydrated sweet potatoes as a concentrate feed for dairy cattle.

REVIEW OF LITERATURE

Feeding trials conducted with fattening pigs (9, 10, 18) indicated that dehydrated sweet potatoes did not produce satisfactory gains, largely due to low palatability and a laxative effect.

For fattening steers, more satisfactory results have been obtained. When sweet potatoes replaced all of the corn in the ration, gains were less rapid than on corn (10, 11, 17) and were less efficiently made (10, 11). In a mixed ration, sweet potatoes were found equal to corn except for the lower protein content (7). In comparison with corn and wheat, gains were as rapid on sweet potato rations but the appraised value was slightly lower (6). When replacing only 50 per cent of the corn, sweet potatoes gave more rapid gains and the selling price on the steers was higher (17).

For dairy cows, Copeland (5) found that dried sweet potatoes were 90.75 per cent as valuable as corn for milk production but that the butter from sweet potato-fed cows had 37.98 I.U. per g. of vitamin A whereas that from the corn-fed cows contained only 31.11 I.U. per g. Trials with dairy cows in Louisiana (14, 16) showed that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal, but are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck.

Digestion trials on dehydrated sweet potatoes have been carried out with steers and lambs (3) and with dairy cows (14, 16). Briggs *et al.* (3) found that with steers the digestibility of the nitrogen-free extract of sweet potatoes was 93.4 per cent when fed with prairie hay and cottonseed meal and 98.5 per cent when fed with alfalfa hay. With lambs the correspond-

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ing values were 87.8 and 92.4 per cent. On a dry matter basis, the total digestible nutrients ranged from 78.7 to 91.4 per cent.

Rusoff *et al.* (14) and Seath *et al.* (16) had difficulty in obtaining the apparent digestibility of fat, fiber, and protein in dehydrated sweet potatoes, but in four trials found digestibility coefficients of 83.47 to 94.46 per cent for the nitrogen-free extract, and calculated total digestible nutrient values from 71.78 to 81.06 per cent on the dry basis. The lower values were for off-grade sweet potatoes which had been cut and bruised and heated to a higher temperature than commonly used.

EXPERIMENTAL PROCEDURE

In this experiment two methods were used to determine the feeding value of dehydrated sweet potatoes. First, a 75-day trial was conducted to determine the milk-producing properties relative to ground yellow corn. Secondly, a digestion trial was conducted to determine digestibility of the main components in order to estimate the total digestible nutrients for more direct comparison with other carbohydrate feeds.

Milk production trial. Four groups of three Holstein cows each were used in the milk production trial. The cows had been fresh from 41 to 142 days at the beginning of the trial; the cow which had been fresh for only 41 days had reached her peak production. These were grouped according to age (six mature cows and six first-calf heifers) and according to milk production within the age groups. Three rations were fed as indicated below. After an equalization and standardization period of 2 weeks, the cows were placed on the experiment, which consisted of three periods of 25 days each, of which the first 5 days was a transition period and the remaining 20 days the experimental period.

The animals were fed alfalfa hay and corn silage at a rate of approximately 8 lb. of hay and 24 lb. of silage per 1,000 lb. of body weight. Grain was fed at first at rates based on the grain feeding table (Appendix Table IXa) in *Feeds and Feeding* (13) for cows receiving the 1.5-lb. hay equivalent, but was adjusted so as to maintain production at approximately the level previous to adjustment. More grain was allowed those animals in their first lactation to allow for growth requirements. Concentrate allowances ranged from 11.6 to 21.0 lb. per day.

Following the suggestion of Lucas (12), the amount of concentrates for all cows was reduced at a rate uniform for all animals regardless of the ration received. As the decrease in production was very slight after the first 2 weeks, no adjustment was made until the end of the first feeding period, at which time the concentrate allowance was reduced 1.5 per cent. The same reduction was made at the end of the second period.

Three rations were used. Ration A consisted of ground shelled corn and soybean meal in a ratio of 650 lb. ground corn to 125 lb. soybean meal.

Ration *B* consisted of 325 lb. ground corn, 325 lb. dehydrated sweet potatoes and 125 lb. soybean meal. Ration *C* consisted of 650 lb. dehydrated sweet potatoes plus 125 lb. soybean meal. This proportion of soybean meal was calculated as supplying sufficient protein to the cows on ration *C*, and some excess to those on rations *A* and *B*. Because of some difficulty in grinding the dehydrated sweet potatoes with the high moisture content when received, the sweet potatoes were fed as they came from the bag, in shreds about 0.25-inch square by 1 to 2 inches long. For cows on ration *B* the sweet potatoes were weighed separately, and the corn and soybean meal mixed in the proper proportion were weighed separately. For cows on ration *C*, the sweet potatoes and soybean meal were weighed separately.

The sweet potatoes used in dehydration were principally, the Maryland Golden variety, but small amounts of Jersey-type sweet potatoes were mixed with them. They consisted of the culls, such as the jumbos, small sized, cut and bruised sweet potatoes. All were cleaned and free of decay.

The ration sequences were arranged according to the method of Cochran

TABLE 1
Organization of milk production trial showing ration sequences

Period	Groups I and III			Groups II and IV		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
I	A	B	C	A	B	C
II	B	C	A	C	A	B
III	C	A	B	B	C	A

et al. (4) to allow for measurement of carry-over effects if these should persist beyond the 5-day transition period. The sequences were arranged as shown in table 1.

Within each group, the particular sequences were assigned to the cows at random.

Milk weights were recorded for each milking. A 1-day composite was accurately prepared once in each 5-day subperiod for a Babcock test. On the last day of each main period a carefully composited sample of milk was prepared for butterfat test, carotene and vitamin A analysis. For the carotene and vitamin A analysis, the method of Boyer *et al.* (2) was used with slight modification. After the extract was concentrated, it was passed through a small chromatograph of sodium carbonate (Frank W. Kerr Co., Detroit, Mich.). Carotene and vitamin A then were determined as recommended. At the same time, blood samples were drawn and the plasma analyzed for carotene and vitamin A according to the method of Boyer *et al.* (1), also with slight modifications. Only 5 ml. of plasma were extracted. After precipitation of the carotene-fat mixture, the samples were stored in the refrigerator until the precipitate had clumped, to facilitate filtration. This storage has not been found to reduce the vitamin A.

Body weights were determined at the end of each period by weighing on three successive days.

Digestion trial. Four mature Holstein cows nearing the end of their lactation periods were dried off 3 to 4 months before they were due to calve. Their requirements for maintenance were estimated according to the Morrison standard, with no special allowance for gestation. The amounts of mixed timothy-clover hay necessary to meet these requirements then were calculated. Two of the cows were placed on the hay ration exclusively. The other two cows received one-half of the calculated amount of hay and an equal number of pounds of dehydrated sweet potatoes.

After 7 days on these rations, the cows were placed in the digestion stall room for the digestion trial carried out as described by Eheart *et al.* (8).

TABLE 2
*Analysis of variance of fat-corrected milk, average butterfat test,
and body weight per period per cow*

Source	Fat-corrected milk		Butterfat test		Body weight	
	Degrees freedom	Mean square	Degrees freedom	Mean square	Degrees freedom	Mean square
Period	2	3,395	2	0.120*	2	2,249*
Group	3	122,416**	3	0.113*	3	47,101*
Period × group	6	4,202*	6	0.070	6	821*
Cows within groups	8	9,905*	8	0.196**	8	26,627*
Ration			2	0.045	2	1,599*
Direct (adjusted)	2	14,180**				
Residual (adjusted)	2	774				
Error	12	1,248	14	0.029	14	261
Standard error per cow		35.3 lb.		0.170%		16.2 lb.
Coefficient of variation (%)		4.78		5.00		1.44

* Represents significance at the 5% point.

** Represents significance at the 1% point.

At the end of this period they were removed from the digestion stalls and changed to the other ration; those which had received hay only were cut to one-half the amount of hay and given an equal amount of sweet potatoes. Those which had been receiving hay and sweet potatoes were changed to hay only, in an amount equaling the total feed received previously. After 9 days the cows were returned to the digestion stalls for a second digestion trial. The changes in weight of the animals themselves were small and not significant.

RESULTS

Milk production trial. The results of the analysis of variance of the data obtained on 4 per cent fat-corrected milk, butterfat test, body weight, blood plasma carotene, blood plasma vitamin A, milk carotene and milk vitamin A are presented in tables 2 and 3. This analysis follows that of

TABLE 3

Analysis of variance of blood plasma carotene, blood plasma vitamin A, milk carotene, and milk vitamin A (γ /100 ml.)

Source	Degrees of freedom	Mean square			
		Plasma carotene	Plasma vitamin A	Milk carotene	Milk vitamin A
Period	2	16,088**	80.44**	62.92**	793.29**
Group	3	5,919*	69.47**	24.03	17.78
Period \times group	6	1,940	4.32	43.26*	9.75
Cows within groups	8	8,716**	39.59**	17.15	18.00
Ration					
Direct (adjusted)	2	33,806**	138.12**	111.52**	60.06*
Residual (adjusted)	2	507	10.60	2.86	0.38
Error	12	1,483	7.43	9.96	10.28
Standard error per cow		38.5	2.73	3.16	3.21
Coefficient of variation (%)		15.9	10.7	19.0	19.3

* Represents significance at the 5% point.

** Represents significance at the 1% point.

Cochran *et al.* (4) with the analysis for direct and carry-over or residual effects of the rations except in the case of butterfat test and body weight. These latter items were analyzed according to the usual procedure without breakdown into direct and residual effects. In no case did the residual effects even approach significance. Direct ration effects were significant at the 1 per cent point for fat-corrected milk, blood plasma carotene, blood plasma vitamin A and milk carotene. Direct ration effects were significant at the 5 per cent point for milk vitamin A. Ration effects were significant at the 5 per cent point for body weight but were not significant for the butterfat test.

Because the residual effects were slight and not statistically significant, the mean values actually obtained, without adjustment, are presented. The mean values for the three rations are presented in table 4 with percentage relationships based on ration A as 100.

TABLE 4

Mean values obtained for rations A, B, and C, and percentage relationships based on ration A as 100

	Mean values			Percentage of ration A	
	Ration A	Ration B	Ration C	Ration B	Ration C
4% fat-corrected milk ^a	38.4	37.4	35.1	97.4	91.4
Butterfat test (%)	3.44	3.42	3.34	99.4	97.1
Body weight (lb.)	1115.3	1118.5	1136.7	100.3	101.9
Plasma carotene (γ /100 ml.)	170.3	277.2	279.8	162.8	164.3
Plasma vitamin A (γ /100 ml.)	21.8	26.1	28.5	119.7	130.7
Milk carotene (γ /100 ml.)	13.0	16.9	20.0	130.0	153.8
Milk vitamin A (γ /100 ml.)	13.9	17.0	19.0	122.3	136.7

^a Lb. per cow per day.

Digestion trial. The digestion coefficients and their standard errors and also the average of seven analyses of the components of the dehydrated sweet potatoes used in the trial are given in table 5.

On the basis of the analysis of dehydrated sweet potatoes actually used in the digestion trials, and considering negative digestion coefficients as zero, the total digestible nutrients are 79.0 per cent on a dry matter basis or 69.6 per cent on a 12 per cent-moisture basis. Using the average of the seven analyses as presented in table 5, the total digestible nutrients are 80.0 per cent on the dry matter basis or 70.4 per cent on a 12 per cent-moisture basis.

TABLE 5
*Digestion coefficients and average chemical composition (7 analyses)
of dehydrated sweet potatoes*

Material determined	Digestion coefficients	Average composition (dry basis)
	(%)	(%)
Crude protein	3.19 ± 3.11	4.86 ± 0.14
Ether extract	52.04 ± 5.74	0.82 ± 0.13
Crude fiber	-51.56 ± 21.61	3.25 ± 0.22
Nitrogen-free extract	90.08 ± 0.43	87.56 ± 0.47
Ash		3.49 ± 0.03

DISCUSSION

The results of this experiment are in line with the results of other work with dairy cows, namely that of Copeland (5) and Rusoff *et al.* (14). In the present trial, when sweet potatoes replaced all of the corn, they were found to be 91.4 per cent as valuable, and when they replaced only half of the corn, they were found to be 94.8 per cent as valuable. On the basis of the calculated total digestible nutrient values, the sweet potatoes were 93.2 per cent as high as corn when compared with the values listed by Schneider (15) for corn grain, found by difference when using cattle for test purposes.

The results on carotene and vitamin A were as expected and corroborate the results of Copeland (5). Dehydrated sweet potatoes have special value for maintaining the carotene and vitamin A in the body and in the milk during the barn feeding period when the quality of the hay is poor, especially when no silage is available.

The standard error per cow and the coefficients of variation for the fat-corrected milk, butterfat test, and body weight were about normal for this type of experiment. In the case of blood plasma carotene and vitamin A, and milk carotene and vitamin A, the coefficients of variation were quite high, but the differences were large enough that the large error terms did not prevent obtaining statistically significant differences.

In the digestion trial the digestibility of the most important constituent, nitrogen-free extract, was high and the results with the four animals were quite uniform. The digestibility of protein was low and not significantly different from zero, but may be quite inaccurate due to the small amount present. The digestibility of crude fiber as determined with each of the four animals was a negative value, but the average was not significantly different from zero. Results on crude fiber at Oklahoma (3) and Louisiana (14) also were negative in some cases.

These results would seem to support the theory that with the increase in the amount of readily soluble carbohydrate in the rumen, the digestibility of the fiber actually may be decreased, through selective use by the rumen organisms. With the small amounts of protein, fat, and fiber present in the sweet potatoes, it might be said that the low digestibility is not important. However, since both the protein and fat already are low compared with corn, the low digestibility merely aggravates a shortage which must be made up by supplying other feeds.

The palatability of the sweet potatoes was quite satisfactory. Three of the cows ate up to 17.6 lb. of sweet potatoes per day in addition to 3.4 lb. of soybean meal. For short periods of time, one of these high-producing cows showed a tendency to leave some of the ration.

The only abnormal results of the experiment were obtained with one of the high-producing cows, whose feces became quite watery. Drug treatment failed to clear up the condition. The appetite was normal and the amount of milk produced continued the same. However, during the period when she was receiving the sweet potatoes, the butterfat test dropped well below normal, averaging 2.4 per cent for four tests. This one cow was largely responsible for the lower fat test for the sweet potato ration. After the discontinuation of the experiment, however, this cow remained in a diarrheic condition for quite some time; this might have been expected as she was turned to pasture after the experiment. In the digestion trial the feces did indicate a slightly more laxative effect for the sweet potato ration than the all-hay ration.

In some areas dehydrated sweet potatoes would seem to show definite possibilities in replacing part of the corn or other carbohydrate feed, if the lower protein and fat are taken into consideration in balancing the rations. At certain times of the year and for certain milk producers, the high carotene content has a special value above the value as a carbohydrate feed.

SUMMARY

1. Dehydrated sweet potatoes when fed to dairy cows in a 75-day double change-over experiment were found to have 91.4 per cent the value of ground yellow corn when they replaced all of the corn. When they replaced only half of the corn, they were 94.8 per cent as valuable.

2. In a digestion trial using four mature dairy cows, the main constit-

uent, nitrogen-free extract, was found to have a digestibility of 90.08 ± 0.43 per cent. On the basis of the digestion coefficients found in this experiment, the total digestible nutrient value was found to be 70.4 per cent on a 12 per cent-moisture basis.

3. Dehydrated sweet potatoes were found to excel corn in maintaining a high level of carotene and vitamin A in the blood plasma and milk. Therefore, they sometimes would have special values which would counteract their slightly lower milk-producing value.

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A SIMPLE COLOR TEST AS AN AID IN GRADING FARM-SEPARATED CREAM^{1, 2}

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It is widely recognized by the creamery industry that a simple test to support the organoleptic method of grading cream would be highly desirable. Although the flavor and odor method is the most satisfactory means available for grading cream for butter making, it has several disadvantages when used by cream station operators as a basis of payment to producers. The chief criticisms are the variability in results due to the personal factor and the lack of visual evidence to support the basis of payment. Supplemental tests for mold, sediment, and acidity often are used for evaluating certain quality factors. However, the correlation between the results of these tests and organoleptic quality is lower than is desired. Furthermore, with the exception of the rapid acidity test, the supplemental tests often are too time consuming for practical use in cream stations for establishing the grade of cream before purchase.

Tests for protein and fat decomposition, even in simplified forms, largely are limited to laboratory use. In addition, their individual relationship with organoleptic grade appears to be too limited for general acceptance as a single measure of quality. No single test would be expected to detect the many possible defects contributing to poor quality. Flavor and odor undoubtedly will remain the principal criteria. Nevertheless, in view of the desirability of a rapid test having high correlation with quality as measured organoleptically and which might be used by cream buyers, field workers and inspectors, data are presented on a method devised for this purpose. The procedure is an outgrowth of observations made during the testing of cream for mold by the Parsons' modification (3) of the Wildman methylene blue-borax method (4). In the latter test it was noted that high quality cream often produced a light colored mixture while poor cream usually produced a darker shade. This observation prompted the testing of various dyes, indicators, and reagents to develop a procedure in which the color obtained with cream would show a suitably close relationship with quality.

MATERIALS AND PROCEDURE

In developing the procedure, the primary objectives were to attain accuracy and simplicity, and to utilize as far as possible facilities and equipment commonly available in cream stations or small plant laboratories. These requisites have governed the quantities of cream and reagents used.

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² This study was supported by a grant from Swift and Company, Chicago, Illinois.

Crystal violet solution. After preliminary tests with various dyes and indicators, crystal violet was selected as the most suitable for the test. The most satisfactory concentration in water had an optical density of 0.136³ after further dilution of 1:250 to facilitate reading. With the lot of dye⁴ used in this work the required concentration was obtained by dissolving 0.5 g. in 1 l. of distilled water and adjusting by further slight dilution in accordance with optical density readings. Although the solubility of the dye in water at 26° C. is given as 1.68 per cent, some sedimentation occurs in the 0.05 per cent solution after prolonged standing. It should be agitated before use and for obtaining consistent results in photometer readings. The dye solution at the concentration used was reasonably stable at room temperature. When held stoppered in 100-ml. to 500-ml. quantities away from direct sunlight, it remained satisfactory for use over periods up to 3 months.

Sodium hydroxide. Exploratory trials with the separate reagents used in the methylene blue-borax test and with several other bases showed that the color differences obtained with 0.1 *N* sodium hydroxide were the most closely associated with cream quality variations. This reagent accordingly was used in subsequent work. When kept stoppered, the 0.1 *N* sodium hydroxide remained of satisfactory concentration over a 6- to 8-week period. Since weaker base results in darker tests, occasional checking against 0.1 *N* hydrochloric acid is desirable if there is doubt as to the concentration of the sodium hydroxide.

Color standard. A color standard was used to indicate the line of demarcation between first and second grade cream. It was prepared to have the same color value as the majority of tests on cream of borderline quality (between first grade and second grade). Under the conditions of the test, and in accordance with the cream grade standards generally accepted in the Kansas area, this color value was near that commonly termed "Iris". Under a daylight-type fluorescent light it was similar to the color value designated as 43-6B in the "Dictionary of Color" (2). The color was reproduced by experimental mixing of white, red, and blue artists' oil paints. It then was applied to the outside of the lower half of a test tube (outside diameter 15 mm). Several tubes were prepared with slight variations in shade and intensity, so that, when dry, the one most closely representing the desired color under a daylight-type fluorescent lamp could be selected. For protection of the dried paint the tube was inserted into a larger test tube (inside diameter 16 mm.) and held in place with a cotton plug and

³ A Coleman spectrophotometer, model 11, and a 0.5-cm. absorption cell were used. Readings were made at 580 mμ, at which setting the dye exhibited maximum absorption with this instrument.

⁴ Distributed by Coleman and Bell Company, Norwood, Ohio. Cert. No. CC 10. Dye content 89 per cent.

cork stopper. The standard was kept in a pasteboard tube when not in use to minimize the possibility of fading. A 2-oz. sample jar painted on the lower half of the inside also was used occasionally as a standard.

Comparator box. Experimentation showed that comparisons of color value could be carried out best by using a small box, open at the front, as shown in figures 1 and 2. A hole was made in the center of the top side for the color standard tube. The box was made large enough to accommodate a 2-oz. sample jar at each side of the color standard. A blue background intensified differences in shade and gave more satisfactory results than the neutral gray usually recommended for color matching. Color

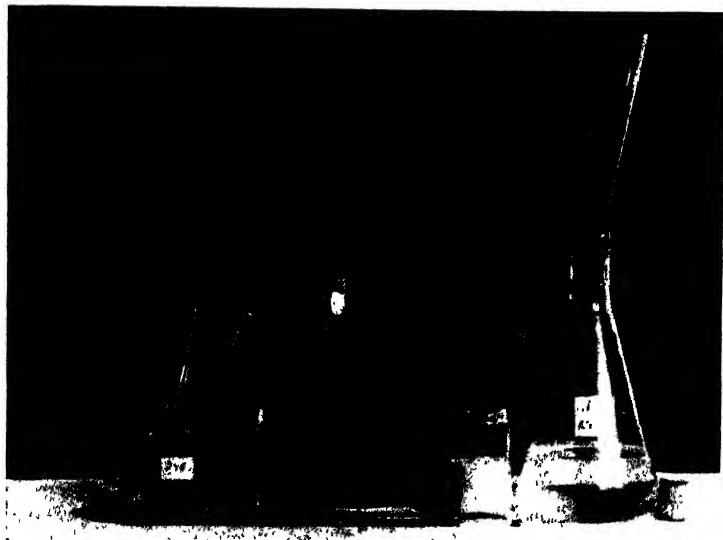


FIG. 1. Materials used for grading cream by the color test.

comparisons on tests were made by placing the box with tests so that light would shine in without shadows.

Light source. The light used for reading the tests affects the color comparison. Apparent changes in color as a result of different types of illumination are more marked in the tests than in the color standard. Since there is considerable variation in the intensity and the color of light in cream stations and other places where cream is graded, it is desirable to have a source of uniform light that will show consistent colors and emphasize small differences. As with color matching generally, a clear north sky light usually is satisfactory. Where this is not available conveniently, a 15- or 20-watt daylight-type fluorescent lamp which gives a slightly bluish light is desirable.

Glassware (fig. 1). The remaining equipment used for making the

test includes the following: 2-oz. cream sample jars (tall type), 1-ml. pipette, 9-ml. pipette, 17.6-ml. pipette, stirring rod, thermometer, bottles for solutions.

Procedure for making the test. Warm sample at about 100° F. until fluid and mix as for Babcock test, pipette 9 ml. of cream into a 2-oz. sample jar, add 17.6 ml. warm 0.1 *N* NaOH (about 120° F.), stir, add 1 ml. crystal violet dye solution and stir. Compare the test sample with color standard in comparator box under daylight-type fluorescent light or clear north sky light. Since the color fades fairly rapidly with cream of good quality, the tests should be read within 1 to 2 minutes after addition of the dye.

Interpretation. As demonstrated later, the color value obtained in the



FIG. 2. The color test on first grade cream (left) and second grade cream (right) showing comparisons with color standard. Color differences are more obvious in the actual tests than in the black and white illustration.

test is primarily a function of both the acidity and the physical condition of the cream. It is based on the fact that crystal violet gradually is decolorized at pH values above 10.5 and that the depth of color obtained on the addition of dye to cream is influenced by the dispersion of cream constituents, more homogeneous distribution giving lighter shades. As cream deteriorates, such recognized changes as coagulation of casein, concentration of fat, separation of serum and other probable physico-chemical changes result in a less homogeneous medium than fresh cream and cause a deeper color with added dye. The physical change is augmented by the stirring and agitation the cream receives during accumulation under practical conditions of production.

With few exceptions the shade (lightness or darkness) of the test varies with the quality of the cream, being a light color with good cream and considerably darker with poor cream (fig. 2). Since the color standard

was prepared to have the same value as tests on cream of borderline quality (between first grade and second grade), those tests lighter than the standard are first grade and the tests darker than the standard are second grade or lower. The darkness or lightness of the color also indicates whether the quality corresponds with the upper or lower range of each grade. Tests with the same color as the standard indicate cream of borderline quality. Because an insufficient number of commercial cream samples of reject quality were obtained to establish a color value for this type of cream, such a color standard was not prepared. Even without this standard, experience in using the test would enable the operator to decide on the color value associated with unacceptable cream. When a variety of commercial reject quality cream samples could be obtained for comparative purposes, the necessary color standard could be prepared.

Comparison of color tests with organoleptic grades. The test was used on 780 samples of cream during spring, summer, fall and early winter.

TABLE 1
*Variations between organoleptic grades of commercial
cream judged by individual graders in pairs^a*

Series ^b	Total samples graded	Whole grade variations		Borderline varia- tions resulting in different grades		Total grade variations	
		(no.)	(%)	(no.)	(%)	(no.)	(%)
1	85	8	9.4	10	11.8	18	21.2
2	147	16	10.9	8	5.4	24	16.3
3	108	7	6.5	18	16.7	25	23.2
Summary	340	31	9.1	36	10.6	67	19.7

^a Of the two graders for each series, one was the same in all series.

^b Each series represents a different set of samples and a different pair of graders.

Most of the samples represented commercial cream as received at cream stations and creameries. The remainder were experimental samples. The result of the test on each sample was compared with the organoleptic grade. Most of the samples were graded by pairs of graders, with each individual working independently. The remainder of the samples were graded by one grader.

RESULTS

Variation between graders when using organoleptic methods. In order to judge the value of the color test for grading purposes, it seemed desirable to have some information regarding the extent of variation usually prevailing between experienced graders on the same cream when grading by flavor and odor. Table 1 gives the results obtained with three series of samples of commercial cream, with two graders for each series, one of whom was the same in all series.

The results show there was disagreement in grades (including borderline differences) in 16.3 to 23.2 per cent of the cases, or an average of 19.7 per cent. Since, under the conditions involved, the graders undoubtedly graded more carefully than they would have done otherwise, this variation probably is less than would be obtained under practical operating conditions. From general observations it is believed that, with graders who have not worked together previously, agreement on grades of commercial cream probably would not be greater than 80 per cent. Accordingly, it is evident that the organoleptic grade of cream is not an absolute measure of quality. It is not an ideal standard by which to measure a proposed test, since differences may be due to inaccuracies in either method. Nevertheless, the organoleptic method is still the most practical, comprehensive and generally-accepted method of indicating cream quality, and state grade definitions are based largely on such an examination. Therefore, it was used as a standard in evaluating the color test described.

Agreement of color test with organoleptic grade. In the 780 samples of cream graded by both the described color test and by organoleptic means, the two methods agreed in 693 or 88.8 per cent of the cases. This compares with the approximately 80 per cent agreement obtained between human graders. Of the 11.2 per cent of the cases where there was disagreement between the color grade and the organoleptic grade, 77 (9.9 per cent of the total) were borderline differences as between a low first grade and a high second grade. Due to the human factors involved in the organoleptic method, there is some question as to whether these borderline differences really indicate inaccuracy of the color test. In only 1.3 per cent of the samples was there disagreement to the extent of a full grade, whereas experienced graders disagreed by a full grade on an average of 9.1 per cent of the samples graded (table 1).

Of the 780 samples which were graded either as first or second grade, 171 or 21.9 per cent were graded as second grade by the organoleptic method while 159 or 20.4 per cent were graded as second grade by the color test method. The color test placed 12 fewer samples (1.5 per cent) in the second grade than did the organoleptic method. This difference, however, is not significant, as indicated by a chi-square test which gave a value of 0.553 with one degree of freedom.

Consistency of the test. In order to determine if the test gave consistent results under similar conditions, 24 different samples of cream were tested in triplicate and comparisons made of the color of the three tests from each sample. No difference could be detected among the triplicates on any of the samples, indicating that the test gave consistent results when conditions were similar. This supported many general observations made during experimental work.

Agreement in reading of color test by different individuals. In order

to determine whether or not differences between individuals in reading the color test would result in significant differences in cream grading, samples from 30 different lots of cream delivered to cream stations by producers were tested. They then were read independently by three individuals using the same light source. Two of the judges had no previous experience in reading the test. The tests were read as lighter, darker, or the same as the standard, corresponding to first grade, second grade, and borderline quality cream. A chi-square test devised by Friedman (1) for ranked data was used to test the agreement among the three judges on the 30 samples. The chi-square was 1.52 with two degrees of freedom; hence it was concluded that the agreement among these individuals in reading the test was entirely satisfactory. Accordingly, since the procedure involved is simple, it is considered that the test is applicable for cream grading, even by inexperienced individuals.

Factors Determining the Color Value Obtained in the Test

As previously stated, the color value obtained in the test is governed principally by the acidity and physical condition of the cream.

Cream acidity. Although crystal violet is not usually considered to be an indicator, it is decolorized at pH values above 10.5. The change is slow at values between 11 and 12 but is more rapid with increasing alkalinity. Accordingly, in the test as applied to cream, one of the principal factors governing the depth of color obtained is the excess alkalinity after the addition of the NaOH, which is influenced by the acidity of the cream. With cream that is almost sweet (0.2–0.3 per cent titratable acidity), the color of the test is light at the start and fades relatively fast. With high acid cream (either from added lactic acid or natural development) the color is much darker and fading is slower. The color differences between the pale hue obtained with fairly sweet cream and the dark shade obtained with high acid cream correspond to a wide range of alkalinity in the tests and generally represent a titratable acidity range in cream as wide as usually is encountered under practical conditions. Hence the test is a partial measure of cream acidity.

Physical condition of the cream. The influence of the physical condition of the cream was demonstrated by the fact that partially churned cream gave darker color tests than the same cream before agitation. In other trials occasional stirring of cream during holding in the laboratory resulted in darker tests than when no stirring was used, even though the final acidity of the stirred and the unstirred cream was practically the same. The effect of the physical condition was further illustrated by using a laboratory hand homogenizer to redisperse the constituents in old, low quality cream. Such treatment presumably resulted in a more homogeneous medium, similar to fairly fresh, good quality cream. The results of

six trials with sweet cream, sweet cream plus lactic acid, and old, low quality cream are given in table 2.

With sweet cream, homogenization gave no observable difference in the color test after most of the air incorporated had an opportunity to escape. When lactic acid was added to the sweet cream, the color test was darker. Homogenization of the cream with added acid caused only a slightly lighter color. This would be expected where the depth of the color was largely the result of acidity rather than of various physical changes in the cream. With second grade cream, however, the situation was different. Homogenizing the cream produced a much lighter color than was obtained in the test on the unhomogenized cream. This would indicate that the depth of

TABLE 2
Effect of homogenizing the cream (in NaOH) on the color grade

Sample no.	Description of cream	Titratable acidity	Color grade	
			Homogenized ^a	Not homogenized
		(%)		
1	Sweet	0.23	1 + ^b	1 +
2	Sweet		1 +	1 +
3	Sweet + lactic acid	0.70	1	1
4	Sweet + lactic acid	0.79	1 -	1 -
5	Second grade cream	1.10	1 -	2 -
6	Second grade cream		1	2

^a In homogenizing, air is incorporated and influences the color somewhat. Hence color comparisons were made after most of the air had an opportunity to escape.

^b + indicates upper range of grade and - indicates lower range of grade.

color was influenced partly by acidity and partly by physical dispersion of the cream constituents.

Although the color obtained in the test is related independently to both the acidity and physical condition of the cream, the combined effect of these two factors gives results more closely related to organoleptic quality than is produced by either factor alone. The fact that the influence of acidity sometimes is modified by the influence of physical condition and vice versa apparently is the reason for the relationship under practical conditions. Examples are presented in table 3 to show that although the color grade generally is associated with acidity, there are exceptions. These exceptions particularly are evident in samples of high acid cream that were of clean flavor and smooth texture. Such samples were of higher organoleptic quality and showed higher color grades than indicated by titratable acidity. On the other hand, some cream samples of lower acidity were also of low quality as shown by both organoleptic tests and the color tests. Such variations between organoleptic grade and cream acidity generally are recognized, and in this respect the color test is in accord with the organoleptic method.

Many other observations made while using the test indicated that the physical condition of the cream (probably associated with the physico-chemical condition) modified the color obtained in the test. Smooth, clean, high-acid cream often graded higher by the test than did other cream of lower acidity but which was grainy, curdled, partially churned, or had other physical characteristics usually associated with low quality cream. In this characteristic the test agrees with recommended grading practices.

Fat content of cream. Tests on sweet cream of 35 to 40 per cent fat content showed very little difference in color from tests on the same cream diluted to 20 to 25 per cent fat with skim milk. The same dilution with water caused a slightly darker test. Apparently variations in fat content

TABLE 3
Variations between titratable acidity and grade of cream

Sample no.	Titratable acidity	Color grade	Organoleptic grade	Remarks
1	0.20	1 +	1 +	
2	0.51	1 +	1 +	
3	0.53	1	1	
4	0.53	1 -	1 -	
5	0.61	2 +	1 -	Thin, watery
6	0.65	1	1	
7	0.68	1 +	1	Clean
8	0.73	1 -	1	
9	0.73	2	1 -	
10	0.91	2 +	2	
11	1.08	2 +	2 +	
12	1.10	2 -	2	
13	1.15	1 -	1 -	Clean, smooth, high acid
14	1.15	2 +	2	
15	1.20	1 -	1 -	Clean, smooth, high acid
16	1.24	2	2 -	

+ indicates upper range of grade and - indicates lower range of grade.

within the range commonly encountered under practical conditions have only a minor influence on the color obtained in the test. That the depth of color obtained is not dependent primarily on the fat content also is shown by the fact that lots of the same cream will give different color tests when held under different conditions resulting in quality variations.

Amount of cream in sample. Even with warm fluid cream there is some variation in the amount of cream measured from different samples. Observations where weighed samples were compared with measured samples and where measured amounts were varied by 0.5 ml. showed that such differences in size of the sample had little influence on the color obtained in the test.

Color and thickness of glass in sample jars. Comparisons indicated that differences in the common 2-oz. sample jars did not cause observable differences in the apparent color of the test.

DISCUSSION

It is unlikely that organoleptic grading of cream, where carefully applied, will be satisfactorily replaced by other tests. However, in circumstances where it has been difficult to promote cream grading, where quality is questionable and where such grading is most needed, it is evident that some simple cream grading aid is desirable. It is considered that the test described has merits in this respect. Although the test was studied under Kansas conditions and standardized to the quality standards and grades in that state, it easily could be adapted to conditions existing in most of the Middle-west area where cream stations are common.

With the exception of the color standard, the test utilizes simple, readily available equipment and is rapid and easy to operate. Although laboratory facilities are necessary for their preparation, the reagents are sufficiently stable for use over a period of several months if kept stoppered. Accordingly, if the reagents and equipment were assembled in the form of a field kit, subsequent operation of the test in stations or factories would be simple.

With few exceptions, the test showed a marked difference in color between good and poor cream. In areas and during seasons when the main cause of low cream quality is deterioration, the test should be useful for grading and in visually demonstrating to cream buyers and producers the different quality grades of cream. The fact that the color value obtained in the test, although influenced by acidity, also is modified by the physical condition of the cream is advantageous. Under practical conditions of production and marketing farm-separated cream, there seems to be a close relationship between the age and quality of cream and its physical condition. Presumably this is due partly to the fact that the longer it takes to accumulate, the more stirring or agitation the cream receives. Also, with increase in age and acidity, the physical or physico-chemical condition undoubtedly is modified, as evidenced by easier churning or whipping. Even at fairly low temperatures there is some separation of fat and serum. Although such changes may not always be evident to the eye, they sometimes are emphasized by the tendency of the cream to oil off on the addition of hot water. General recognition of the relationship between quality and physical condition of cream is indicated in the cream grading laws of several states, which stipulate that first grade cream must be smooth and free from lumps, and that lumpy, curdy cream is second grade. The characteristic of the color test of grading down the latter type of cream is in accordance with recommended grading practices.

Where state cream grade definitions place an acidity limit on grades, it would seem that a rapid acid test often would be a necessary complementary test to any other grading method. Although the described color test correlates fairly well with acidity, and the color standard could be modified to compare with desired acidity limits, other factors associated

with quality also are measured by the test. In areas where cream quality generally is high and deterioration is a relatively minor factor compared with other flavor defects (absorbed, weed, feed, etc.), the test would be expected to show less correlation with quality measured organoleptically.

SUMMARY AND CONCLUSIONS

1. A simple color test as an aid in grading farm-separated cream at time of purchase is described. Instructions for the preparation of reagents, description of equipment needed and details for the testing procedure are given.

2. Although the preparation of reagents and the color standard requires laboratory facilities, the operation of the test is simple and is particularly suitable for field work and cream station conditions.

3. The test is based on the depth of color resulting from the addition of 17.6 ml. of 0.1 *N* NaOH and 1 ml. of crystal violet dye solution of given concentration to 9 ml. of cream in a 2-oz. sample jar. Under the conditions of making the test, the color value obtained primarily is related to the acidity and the physical condition of the cream. Under practical conditions of production the combined acidity and physical condition of cream appear to correlate closely with organoleptic grade.

4. Comparison with a prepared color standard of the color value obtained on cream tests permits cream to be graded as first or second grade, and also usually indicates whether it falls in the upper or lower range of the grade.

5. The test was used on 780 samples of experimental and commercial cream from stations and creameries during late spring, summer, fall and early winter. Agreement with organoleptic grades was obtained to the extent of 88.8 per cent of the cases. Most of the differences were only borderline variations. In only 1.3 per cent of the samples did the difference represent a whole grade. This was closer agreement than was obtained between organoleptic grades as judged by experienced graders.

6. It is expected that the test would be most applicable in those areas where poor cream quality is largely the result of deterioration rather than of flavor defects of other types. Since the test is a partial measure of acidity, the color standard could be modified so that the test would be useful even in states where specific acidity limits are placed on cream grades.

7. From the results obtained it appeared that the test offers a means of promoting cream grading in localities where little grading is practiced and where general improvement in quality is needed.

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THE EFFECT OF PREPARATION OF THE COW ON THE RATE OF MILKING^{1, 2}

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One of the important jobs in the management of a dairy herd is that of milking. Several workers have reported on various aspects dealing with this problem. Studies by Zwart (11) and Tgetgel (10) showed a gradual rise in udder pressure from one milking to the next. Gaines (6), Tgetgel (10) and Krzywaneck and Brüggemann (8) noted marked increases of intraglandular pressure after mammary stimulation.

Gaines (6, 7), Espe (4) and Foot (5) observed latent periods of varying lengths after stimulation before a "let-down" or excretion of milk occurred.

Elting and LaMaster (2) studied the effect of foremilk on the rate of mechanical milking. They reported that foremilk increased the rate of milk flow in the earlier part of the milking process but prolonged the time required for stripping. The over-all effect was an increase in the total time required for milking. Dodd and Foot (1) found that stimulation before milking shortened the time required for milking. The object of this experiment was to ascertain the effect of preparation or obtaining a let-down of milk before attachment of the milking machine on the rate of milk withdrawal.

EXPERIMENTAL PROCEDURE

Four 2-year-old Holsteins, E401, E405, E413 and A55, and one of mixed breeding, E408, and a 5-year-old grade Holstein, A30, were used in this study. Cows E401 and E405 were hand milked before the beginning of this experiment. Cows E408, E413 and A55 were milked by machine beginning 3 days after calving. A30 was in her third lactation and had been milked by machine in previous lactations.

During the course of the experiment the cows were milked twice daily at 12-hour intervals. At the evening milking of one day and the morning milking of the following day, the cows were stimulated before milking. At the successive evening and morning milking the cows were not stimulated previous to milking. The cows were stimulated to let down or excrete

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milk by a 10- to 15-second wash and massage of the teats and udder with water at a temperature between 120 and 130° F. 2 minutes before the milking process began. Before attaching the teat cups, each quarter was fore-milked by expressing two streams of milk from each teat. For the non-stimulated milkings, the teat cups were merely attached without prior washing or massaging of the teats or udder.

The milking machine was suspended from a scale and readings were taken every 10 seconds from the time the last teat cup was put on until the "end-point" of milking was reached. For the purposes of this study the end-point of a milking was taken as the time after milking when the increment in yield of three successive 10-second scale readings was three-

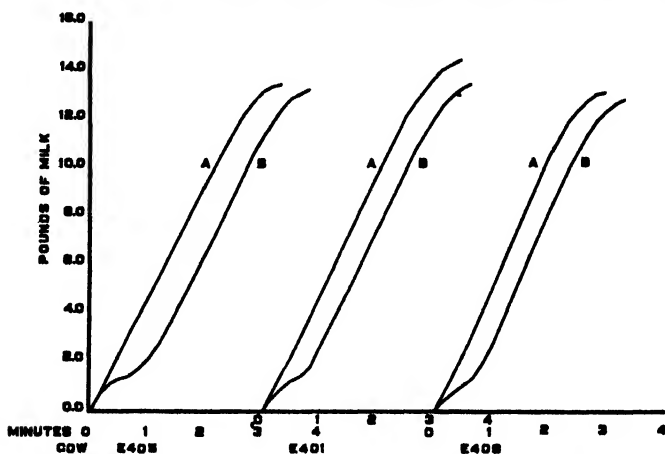


FIG. 1. The effect of stimulation and non-stimulation on the shape of the milk curve. A—Stimulated; B—Not stimulated.

tenths of a pound or less, or when two successive readings were identical. The criterion used depended on which occurred first. The use of an end-point was justified by the fact that preliminary readings had shown that the rate of flow was practically constant to the end-point and machine stripping should occur at that stage of the milking process. A stop watch was used for timing purposes. Ten milkings were recorded for each régime. The milking machine used was a double-action, constant-vacuum type and was operated at 15 inches of mercury negative pressure, commonly called vacuum, on the line and 50 pulsations per minute. There was a difference of 0.5-inch mercury negative pressure between the line and milk hose when milk was not flowing.

RESULTS

Table 1 presents the results of this study. Figure 1 shows the milk curve for three of the cows. A lapse of 30 to 60 seconds occurs before the

TABLE 1
Mean accumulative total pounds of milk for ten milkings when the cows were stimulated and not stimulated before attaching the milking machine

Milking time (minutes and seconds)	Cow E405		Cow E401		Cow E408		Cow A30		Cow A55		Cow E413	
	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated
0: 10	(lb.) 0.71	(lb.) 0.65	(lb.) 0.71	(lb.) 0.48	(lb.) 0.65	(lb.) 0.41	(lb.) 0.98	(lb.) 0.56	(lb.) 0.58	(lb.) 0.32	(lb.) 0.78	(lb.) 0.53
0: 20	1.52	1.08	1.46	0.86	1.39	0.73	1.75	0.77	1.21	0.70	1.55	1.16
0: 30	2.33	1.29	2.21	1.15	2.22	0.98	2.52	0.88	1.85	1.09	2.23	1.66
0: 40	3.18	1.43	3.03	1.42	3.08	1.30	3.38	1.10	2.49	1.47	3.28	2.09
0: 50	3.95	1.65	3.88	1.83	3.99	1.90	4.20	1.62	3.15	2.03	4.16	2.77
1: 00	4.73	2.08	4.71	2.56	4.89	2.84	5.04	2.36	3.84	2.63	5.06	3.55
1: 10	5.51	2.70	5.53	3.36	5.82	3.52	5.89	3.23	4.53	3.28	6.00	4.46
1: 20	6.28	3.43	6.38	4.18	6.75	4.41	6.74	4.12	5.21	3.93	6.89	5.30
1: 30	7.04	4.20	7.21	5.01	7.70	5.36	7.63	5.03	5.88	4.61	7.81	6.19
1: 40	7.86	4.98	7.98	5.84	8.65	6.29	8.48	5.91	6.54	5.23	8.65	7.03
1: 50	8.63	5.79	8.90	6.63	9.52	7.19	9.30	6.78	7.16	5.79	9.46	7.84
2: 00	9.40	6.54	9.68	7.45	10.36	8.10	10.15	7.64	7.70	6.52	10.19	8.68
2: 10	10.18	7.38	10.52	8.27	11.08	9.02	10.94	8.53	8.42	7.17	10.94	9.47
2: 20	10.94	8.14	11.33	9.09	11.75	9.86	11.65	9.43	9.02	7.82	11.67	10.15
2: 30	11.64	8.95	12.08	9.93	12.32	10.64	12.34	10.28	9.62	8.42	12.43	10.87
2: 40	12.27	9.79	12.69	10.74	12.74	11.32	12.93	11.06	10.15	9.07	13.15	11.57
2: 50	12.77	10.57	13.22	11.35	12.99	11.87	13.45	11.77	10.60	9.69	13.77	12.59
3: 00	13.17	11.30	13.69	12.04	13.12	12.31	13.95	12.44	10.97	10.21	14.24	12.97
3: 10	13.44	11.91	14.06	12.60		12.59	14.33	13.02	11.28	10.71	14.53	13.60
3: 20	13.62	12.46	14.33	13.03		13.75	14.69	13.55	11.54	11.16	14.71	14.14
3: 30		12.87	14.50	13.32			14.87	14.02	11.71		14.70	14.51
3: 40		13.10		13.46				14.37			14.37	14.70
3: 50		13.27						14.70			14.82	14.82
4: 00								14.85				
Total milk obtained	14.63	14.76	14.97	15.03	14.53	14.53	16.58	16.16	13.05	13.40	15.67	15.95
Per cent total at end point	93.1	89.9	96.9	89.6	90.3	87.7	89.7	91.9	89.7	91.7	93.9	92.9
Mean rates per min.	4.08	3.48	4.14	3.66	4.38	3.84	4.26	3.72	3.36	3.06	4.44	3.72

milk is let down when the cows are not stimulated, as evidenced by examination of the table and the plateaus in the curves. The curves of the rate of milk removal are markedly different in shape when the cows were stimulated and not stimulated before attaching the milking machine.

All six cows had appreciably higher mean rates of removal of milk when stimulated before milking. The rates of milk flow after stimulation for cows A30, A55, E413, E405, E401 and E408 were 0.71, 0.56, 0.74, 0.68, 0.69 and 0.73 lb. per 10 seconds, respectively, whereas the rates for non-stimulation of the cows in the same order were 0.62, 0.51, 0.62, 0.58, 0.61 and 0.64 lb. per 10 seconds. From 10 to 30 seconds less time was required to reach the end-point when the cows were stimulated, and only with cows A30 and A55 was the percentage of the total at the end-point slightly lower than when not stimulated.

A30 was milked for a period of 15 consecutive days to test the response to continuous non-stimulation. The milking machine was operated under the same conditions as in the previous experiment. The mean total at 1 minute for 29 milkings (readings for one milking were missed when the milk hose dropped off) was 2.38 lb. Reference to table 1 shows that the mean total of ten milkings at one minute was 5.04 lb. for A30 when stimulated before milking. The mean total at 1 minute of the continuous non-stimulation milkings was very similar to that of the mean of the ten milkings, 2.36 lb. of non-stimulation when alternated daily with stimulation before milking.

DISCUSSION

A very definite plateau occurred in the milk curves when the cows were not stimulated, as the milk had not been let down and the sinuses were soon evacuated. The results obtained in this investigation are in agreement with the findings of Gaines (6, 7), Foot (5) and Espe (4). Practically a constant rate of flow was obtained from the time the milking process began until the end-point was reached when the cows were prepared for milking by prior stimulation. The initial flow of milk of about a pound when the cows were not stimulated represents the milk that had drained into the large ducts and the gland and teat sinuses.

Ely and Petersen (3) noted a response by the let-down or excretion of milk 45 seconds after the injection of oxytocin. Thus, the plateau in the milk flow curve of cows not prepared for milking by stimulation is the time necessary for the milking stimulus to motivate the posterior lobe of the pituitary to secrete the oxytocic principle into the blood stream and cause a let-down or excretion of milk.

Before stimulation, the teats are soft and flabby, but they become firm and turgid after stimulation as a result of the let-down or excretion of milk, with a resulting increase in intraglandular pressure. It was observed that teat cups are much more easily attached to a turgid than a flabby teat.

Petersen (9) reported that the teat cups crawl or draw in the slackened udder tissue when the intraglandular pressure is low and occlude the orifices between the gland and teat sinuses. The results of this study show that the time required for milking was longer when the cows were not stimulated. Obviously a milking machine attached to the teats of an udder in which an increment of intraglandular pressure has not been effected by stimulation may occlude the passage between the gland and teat at the beginning of milking, thereby prolonging the milking process. In addition, when the sinuses have been drained, as represented by the plateau in the milking curves, the teat cups draw in the flaccid udder tissue and trauma may result to the secretory tissue at the juncture of the teat and gland.

SUMMARY AND CONCLUSIONS

Preparing the cow for milking by stimulation with a wash and massage of the udder with water at 120 to 130° F. was found to increase the rate of milking and decrease the time required for the milking process as compared with no preparation.

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THE REDUCING CAPACITY OF MILK AND MILK PRODUCTS AS MEASURED BY A MODIFIED FERRICYANIDE METHOD^{1,2}

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In 1945 Chapman and McFarlane (5) published a method for determining reducing substances in milk and milk products by heating with potassium ferricyanide under specific conditions. The method was adapted from the procedure used by Anson (1, 2), Mirsky (12) and Mirsky and Anson (13) for determining sulfhydryl groups in proteins. Chapman and McFarlane found that fresh milk possesses considerable capacity to reduce ferricyanide under the conditions used and that heat treatment of milk or storage of milk powder open to the atmosphere increases the reducing capacity.

In 1945 Harland and Ashworth (8) reported the use of thiamin disulfide for estimation of the reducing power of milk. This reagent evidently is a much weaker oxidant than ferricyanide, at least under the conditions used, since it is not reduced at all by normal unheated milk. Heat treatment, however, does produce materials which reduce thiamin disulfide but long continued heat treatment in the presence of air causes a subsequent decrease in reducing power. The disparity in behavior of milk to these two reagents prompted Chapman and McFarlane (6) to express the opinion that these reagents react with different reducing systems. This opinion is somewhat substantiated by the work of Lea (10), which indicates that materials produced by interaction of lactose and protein are responsible for the increase in reducing capacity of dry milk during storage at 47° C. and 55 per cent relative humidity.

The work reported in this paper was undertaken prior to publication of Lea's results to examine the ferricyanide method and to determine which constituents of milk reduce this reagent and contribute to the increases produced by processing and storage.

METHOD

Factors affecting reduction of ferricyanide. The extent to which ferricyanide is reduced by a system such as milk is strongly influenced by the

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hydrogen ion concentration, which determines the reduction potential of the several reductants present. Furthermore, since the reaction is slow, it usually is not allowed to go to completion. Hence the temperature and time of the reaction become of great importance in determining the extent of reduction. Chapman and McFarlane (5) made some study of the effect of the three variables—pH, temperature, and time—on the amount of ferricyanide reduced by milk powder. They showed: (a) that the capacity to reduce ferricyanide increases markedly from pH 2 to pH 8, (b) that the reaction proceeds much faster at 70° C. than at 50° C. (at pH 5.0),

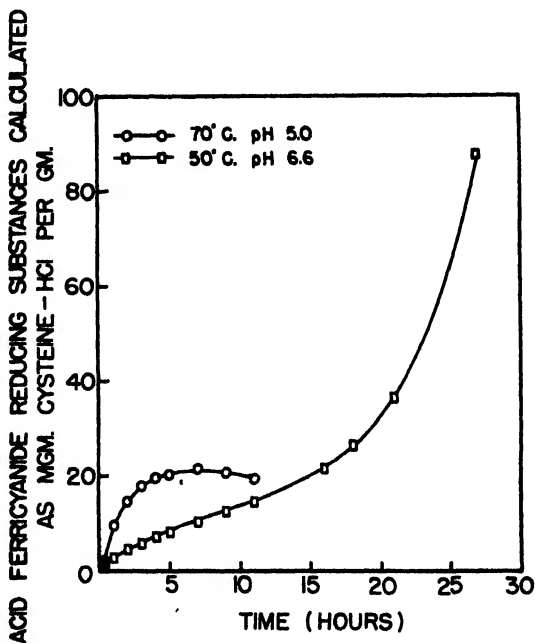


Fig. 1. Reduction of ferricyanide by dry whole milk.

and (c) that the reaction apparently is far from complete in 40 minutes (at 70° C. and pH 5.0). Others (13) have shown that protein continues to reduce ferricyanide for 12 to 24 hours at least. On the basis of their study of the factors influencing the reduction, Chapman and McFarlane (5) adopted the standard conditions of pH 5.0, 70° C., and 20 minutes as giving a satisfactory differentiation between fresh and aged samples.

In employing the method of Chapman and McFarlane, the present authors soon encountered a serious difficulty. In some cases, particularly with powders of high reducing capacity, a blue precipitate was retained on the filter paper when the reaction mixture was deproteinized. This phenomenon could be attributed to partial decomposition of the ferricyan-

ide during heating with liberation of ferric ions which react with ferrocyanide to form Prussian blue (ferric ferrocyanide). Since the extent of reduction is determined by formation of Prussian blue after deproteinization, any such removal of ferrocyanide by "preformation" of Prussian blue might seriously vitiate the results. Consequently, conditions which would obviate this difficulty were sought for conducting the reaction.

It soon was found that by raising the pH to 6.6 and lowering the temperature to 50° C., no Prussian blue was preformed, although the rate of

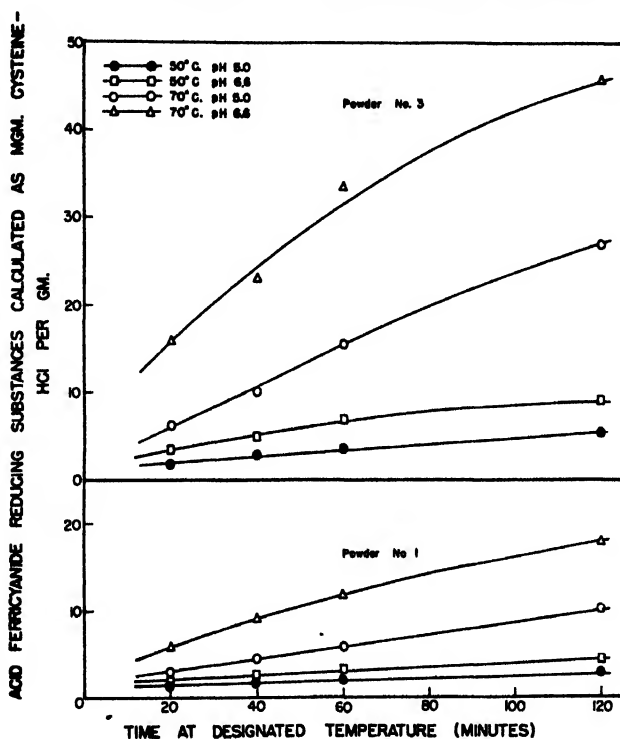


FIG. 2. Effect of temperature and pH on rate of reduction of ferri-cyanide by two samples of dry whole milk.

reduction was somewhat lower than that at pH 5.0 and 70° C. Figure 1 shows a comparison of the rate of reduction at pH 5.0, 70° C., with that at pH 6.6, 50° C. The fact that the reduction at pH 5.0, 70° C. reaches a maximum and even tends to drop off is attributable to the "preformation" of Prussian blue. Evidently, reduction continues indefinitely at pH 6.6, 50° C. Figure 2 shows the rates of reduction of two powders under the following conditions: pH 5.0, 70° C.; pH 5.0, 50° C.; pH 6.6, 70° C.; and pH 6.6, 50° C. Of these four, the last yielded satisfactory differentiation

without the use of excessively high temperature; consequently, it was adopted, together with a standard reaction time of 20 minutes.

Chapman and McFarlane (5) stated that the use of 5 ml. of 1 per cent ferricyanide per 100 mg. of milk powder yielded maximum color intensity. In the present study limited data indicate that greater intensities are obtained by increasing the concentration of ferricyanide. However, this point has not been investigated very extensively, and 5 ml. of 1 per cent solution have been employed routinely.

Folin (7) and Anson (1) have indicated that impurities may be encountered in ferricyanide and have suggested methods for purification. Furthermore, Anson (1) advised storage of ferricyanide at 5° C. in the dark and checking it occasionally for the presence of ferrocyanide. In the present study no evidence of impurities in reagent grade ferricyanide was observed, but solutions of it did deteriorate at room temperature. No evidence of deterioration of solutions stored in the dark at 5° C. for periods up to 15 days has been found.

Factors affecting color intensity. Chapman and McFarlane (5) stipulated the use of "freshly prepared" ferric chloride solution for formation of Prussian blue in the deproteinized filtrate. In the present study, holding such solutions for periods up to 4 days was practically without effect on color intensity, but the general rule of preparing fresh solution each day was adopted.

The procedure of Chapman and McFarlane (5) in reading the color intensity at exactly 10 minutes after addition of the ferric chloride solution was followed. Under these conditions, of course, the color intensity does not follow Beer's law and a calibration curve must be used. Lea (10) has reported that, if the holding period is limited to one minute, Beer's law is obeyed, but in the opinion of the present workers any advantage gained by such a procedure is offset by the fact that slight variations in holding time would introduce larger errors than in the case of the 10-minute holding period.

The method adopted. Weigh a 100-mg. sample of dry milk or simplified system into a test tube (22 × 150 mm.) and disperse it in 5 ml. of distilled water at 50° C. Alternatively reconstitute 5 g. in 250 ml. of distilled water and use a 5-ml. aliquot. Add 5 ml. of a buffer at pH 6.6 (M/5 potassium dihydrogen phosphate and M/5 sodium hydroxide) and 5 ml. of 1.0 per cent potassium ferricyanide. Mix well and heat for exactly 20 minutes in a continuously agitated water bath maintained at 50° C. Cool immediately to 25° C. or lower in an ice water bath. Add 5 ml. of 10 per cent solution of trichloroacetic acid, mix and filter through no. 40 Whatman filter paper. Transfer 5 ml. of the filtrate to a test tube (22 × 150 mm.) and dilute with 5 ml. of distilled water. Add 1 ml. of fresh 0.1 per cent ferric chloride solution and mix thoroughly by vigorous shaking. If several de-

terminations are being made, add the ferric chloride solution to the tubes at intervals of 1 minute and hold each tube for exactly 10 minutes before determining the color intensity. A pair of matched square cuvettes and a Coleman Universal Spectrophotometer have been used, making all readings at $660\text{ m}\mu$ with the reagent blank set to read 100 per cent transmission. The reagent blank is similar to the unknown except that 5 ml. of water is substituted for the sample.

In some cases it was desired to determine the proportion of the reducing capacity contributed by components of the system other than protein. For this purpose a portion of reconstituted sample was deproteinized with

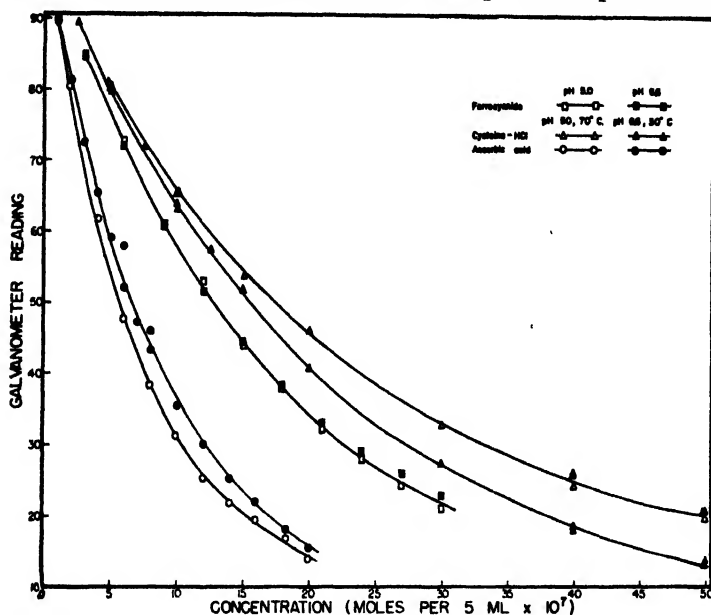


Fig. 3. Calibration curves showing the relation of the intensity of Prussian blue color to concentration of ferrocyanide, cysteine, and ascorbic acid.

tungstic acid and ferricyanide reduction determined in the filtrate. The use of a buffer having a pH of 7.4 was found necessary to insure a final pH of 6.6. The detailed procedure is as follows: Reconstitute 1 g. of powder with 25 ml. of distilled water in a 50-ml. volumetric flask. Add 16 ml. of 0.33 *N* sulfuric acid, 8 ml. of 10 per cent sodium tungstate and sufficient distilled water to bring to volume. Mix thoroughly, hold for 10 minutes and filter. For the ferricyanide reduction use 5 ml. of filtrate (equivalent to 100 mg. of powder) and 5 ml. of buffer at pH 7.4. Proceed from this point exactly as for whole milk except that filtration after adding trichloroacetic acid may be omitted.

Expression and reproducibility of results. The choice of units for expression of the reducing power of milk is complicated by the multiplicity of reductants involved. Faced with this situation, Chapman and McFarlane (5) chose to calibrate the method with glutathione and to express the capacity of milk to reduce ferricyanide in terms of the molar concentration of glutathione sulfhydryl groups required for an equivalent reduction. However, Lea (10) contends, with considerable justification, that, in view of the lack of knowledge of the specific groups involved, it is preferable to express reducing power of milk in terms of moles of ferriyanide reduced.

Figure 3 shows a curve relating galvanometer reading to concentration of potassium ferrocyanide. In obtaining the data for this curve a series of solutions containing 1.0 per cent ferricyanide and concentrations of ferrocyanide up to 60×10^{-5} molar was prepared. Five milliliters of such solution, 5 ml. of buffer (either pH 5.0 or pH 6.6), 5 ml. of water, and 5 ml. of 10 per cent trichloroacetic acid then were mixed and a 5-ml. aliquot taken. To this was added 5 ml. of water and 1 ml. of 0.1 per cent ferric chloride and the color intensity read after 10 minutes. Little if any effect of pH on color development was found. Figure 3 also shows curves relating concentration of cysteine and ascorbic acid to the intensity of blue color obtained from ferrocyanide produced by reduction of ferricyanide by these reductants.

In figure 4, the concentration of ferrocyanide necessary to produce a given color intensity has been plotted against the concentration of cysteine or ascorbic acid which produces an identical intensity by reduction of ferricyanide. Ascorbic acid reacts very nearly stoichiometrically at pH 6.6, 50° C. with ferricyanide; the slope of the line indicates that 1 mole of ascorbic acid reduces 1.95 moles of ferricyanide, which is very close to the theoretical equivalent of 2.00. At pH 5.0, 70° C., slightly more than 2 moles of ferricyanide are reduced by a mole of ascorbic acid. Tauber and Kleiner (14) employed a somewhat similar method for determining ascorbic acid but the reduction was carried out at a lower temperature (40° C.) in a more acid medium (10 per cent trichloroacetic). Their data give no evidence as to the stoichiometry of the reaction. (See also Ball (3).)

The extent of reduction by cysteine is neither so complete nor so uniform over the concentration range studied as is that by ascorbic acid. In the range of concentration from 5 to 32×10^{-7} moles per determination, a mole of cysteine reduced about 0.85 mole of ferricyanide at pH 6.6, 50° C., and about 0.70 mole at pH 5.0, 70° C. Mason (11) estimated glutathione by oxidation with ferricyanide at pH 5.9 at room temperature and determination of Prussian blue. The oxidations of cysteine and glutathione were stoichiometrically equivalent under these conditions, but the paper gives no information as to whether the ferricyanide reduced is stoichiometrically equivalent to the cysteine or glutathione oxidized. However,

Anson (1) obtained 0.00104 mM of ferrocyanide when 0.001 mM of cysteine was oxidized with 0.2 mM of ferricyanide for 20 minutes at pH 6.8, 50° C., which does indicate a stoichiometric relation. While these data support Lea's (10) contention that the reduction of ferricyanide by cysteine is not stoichiometric under the conditions used, they do furnish an empirical relation which could be used in converting from one basis to another.

All results are expressed in terms of equivalent cysteine hydrochloride

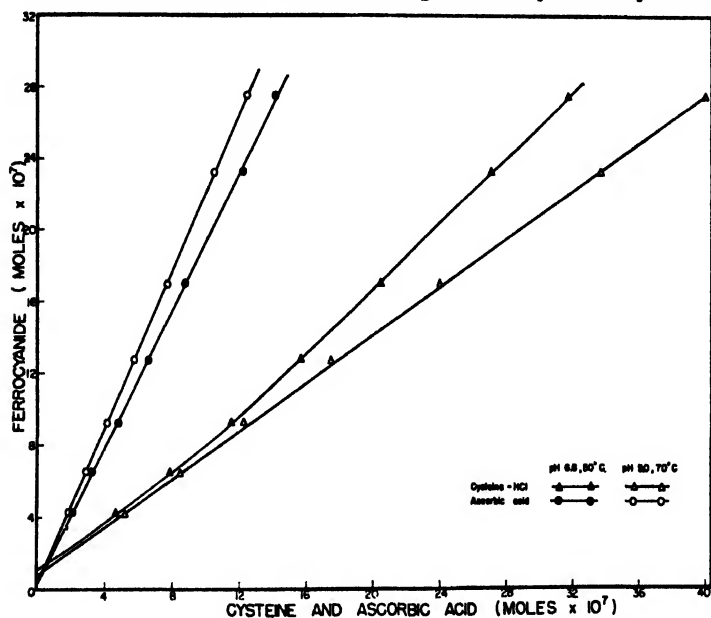


FIG. 4. Relation between cysteine or ascorbic acid oxidized and ferrocyanide produced.

concentrations because such standardization furnishes a common basis for comparing various methods.

To test the reproducibility of results, several 100-mg. samples of two different milk powders were weighed into test tubes which were stoppered and held at -10° C. On several occasions determinations were made on these samples by two individuals working independently. The results, shown in table 1, indicate a rather satisfactory degree of reproducibility for either individual. However, there is a small but consistent unexplainable difference between individuals.

MATERIALS

The materials used in this study were those described in a recent paper on fluorescence (9). Briefly, they were spray dried milk, acid-precipitated

TABLE 1

Comparison of results obtained by two individuals with the ferrioyanide method

Trial ^a	Reducing substances as cysteine-HCl per g.			
	Sample 358		Sample 49	
	A ^b	B ^b	A ^b	B ^b
	(mg.)	(mg.)	(mg.)	(mg.)
1	1.32	1.42	2.88	2.93
2	1.32	"	2.84	"
3	1.36	"	2.95	"
4	1.38	1.41	2.84	3.01
5	1.37	1.45	2.84	2.92
6	1.32	1.41	2.90	2.87
Mean	1.35	1.42	2.88	2.93

^a Each trial made on a different day.^b Individuals designated A and B.

casein, dialyzed milk serum protein, filtered milk fat, a concentrate of fat globule "membrane" from washed cream, and commercial samples of lactose, riboflavin and ascorbic acid.

RESULTS

Effect of processing on the reducing capacity of whole milk. Chapman and McFarlane (5) have shown that an increase in the temperature of preheating the fluid milk increases the acid ferricyanide reducing substances in spray-dried whole milk. The data presented in table 2 indicate that the reducing capacity of the dry whole milk also is influenced by the temperature of spray drying. The use of higher drying temperatures nay, in fact, overshadow the effect of variation in preheating temperature.

TABLE 2

Effect of preheating and spray-drying on acid ferricyanide reducing substances in dry whole milk

Series	Preheat treatment		Reducing substances as cysteine-HCl per g. of solids					
	Temp.	Time	Fresh	Pre-heated	Con-densed	Frozen dried	Spray-dried	
							N ^a	H ^b
	(°C.)	(Min.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
1	66	30	"	"	"	"	1.77	2.55
2	66	30	1.00	0.96	0.96	"	1.20	1.88
3	74	30	0.96	1.00	1.08	1.11	1.24	1.58
4	74	30	1.05	1.08	1.18	"	1.90	"
5	74	30	0.86	0.86	"	"	"	"
6	74	30	"	"	"	1.22	1.63	"
1	85	20	"	"	"	"	1.80	2.47
2	85	20	1.00	1.14	1.09	"	1.80	1.97
4	85	30	1.05	1.21	"	"	"	"
5	85	30	0.86	1.02	"	"	"	"

^a N = Normal drying temperature.^b H = High drying temperature.

As is shown by data in table 2, drying from the frozen state under vacuum is essentially without effect on the reducing capacity of whole milk. This fact has been confirmed in experiments with other samples.

Contribution of the constituents of milk to the reducing effect. (a) Caseinate and caseinate-lactose systems—Casein was dispersed in sufficient lime water to produce a sol at pH 6.6 containing 1.0 g. of casein per 16 ml. of sol. Lactose was added to portions of this sol to produce sols with 0, 0.025, 0.05, 0.10, 0.50, 1.0, 2.15 and 4.30 parts of lactose (weighed as α -hydrate) per part of casein. The effect of heat treatment at various

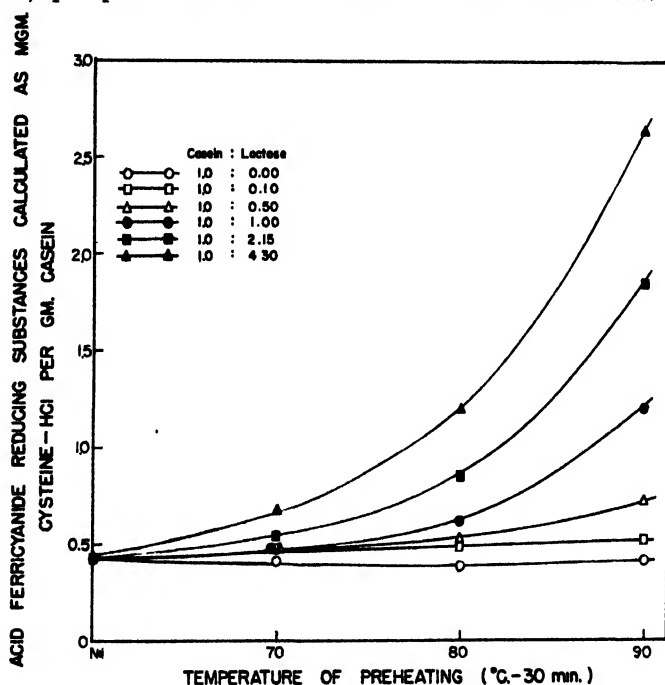


FIG. 5. Effect of heat treatment on acid ferricyanide reducing materials in casein-lactose systems.

temperatures on the acid ferricyanide reducing capacity of these sols is shown in figure 5.

Heat treatment produced no change in the reducing effect of the calcium caseinate sol, but in those sols containing lactose as well as calcium caseinate, there was an increase with increase in temperature, which, at any given temperature, was roughly proportional to the lactose content.

(b) Serum protein and serum-protein-lactose systems—Milk serum protein prepared as described by Jenness and Coulter (9) was equilibrated against phosphate buffer (pH = 6.6, μ = 0.1) and adjusted to a protein con-

centration of 1.0 g. per 100 ml. Lactose was added to give mixtures containing, respectively, 0, 0.164, 1.632 and 7.06 g. of lactose (weighed as α -hydrate) per g. of serum protein. A solution containing 7.06 g. of lactose (α -hydrate) per 100 ml. of the buffer but no protein was included for comparison. The effect of heat treatment at various temperatures on the acid ferricyanide reducing capacity of these systems is shown in figure 6.

In contrast to the effect of heat on the calcium caseinate sol, heating of the serum protein sol resulted in an increase in the acid ferricyanide re-

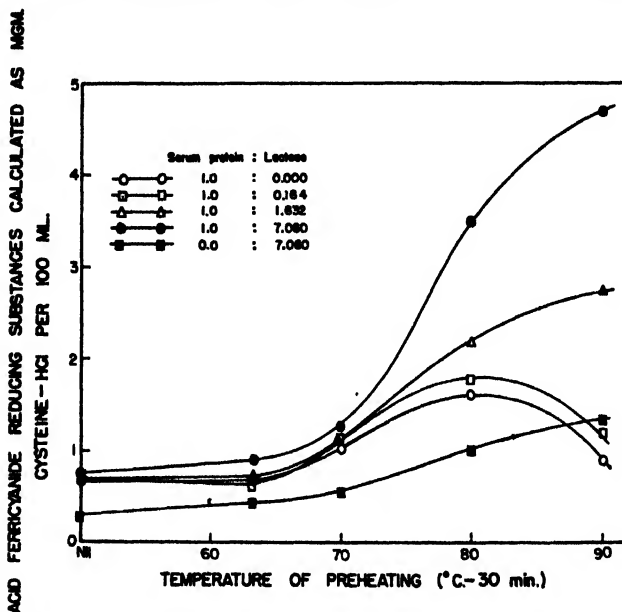


Fig. 6. Effect of heat treatment on acid ferricyanide reducing materials in milk-serum-protein-lactose systems.

ducing effect, but the maximum capacity was not produced by the most drastic heat treatment used. Evidently greater oxidation of reducing groups liberated from the protein occurred on heating at 90° C. Heat treatment of lactose solutions in phosphate likewise produced materials capable of reducing acid ferricyanide. The effect of heat treatment on systems containing both serum protein and lactose was to produce reducing capacities much greater than the sum of those produced from the two constituents separately. These increases were related both to temperature and to the lactose content of the systems.

(c) Systems of calcium phosphocaseinate and other milk constituents dried from the frozen state—As already shown, the drying of whole milk

from the frozen state does not increase its reducing capacity appreciably. The effect of preheating various liquid-simplified systems at temperatures of 74 and of 90° C. for 30 minutes on their reducing capacity after drying from the frozen state is shown in table 3. These systems were prepared as described in a previous paper (9). All of the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of the most complete system (*i.e.*, that containing all of the constituents). Expression of the results in this manner makes immediately apparent the contribution of each constituent to the reducing capacity of the complete system.

The addition of lactose to a caseinate system enhanced both the initial

TABLE 3

Production of acid ferricyanide reducing substances in simplified systems dried from the frozen state under vacuum

System	Constituents ^a	Reducing substances as cysteine-HCl per g. complete system ^b		
		Heat treatment for 30 min.		
		None	74° C.	90° C.
		(mg.)	(mg.)	(mg.)
1	Caseinate ^c	0.05	0.05	
2	1 + lactose	0.14	0.20	0.30
3	2 + serum protein	0.20	0.28	0.35
4	2 + milk fat		0.20	
5	4 + f.g.m. ^d	0.20	0.25	0.38
6	5 + serum protein	0.36	0.39	0.56
7	6 + riboflavin		0.33	
8	7 + ascorbic		0.50	

^a Ratio of constituents was as follows: 1.00 casein: 2.04 lactose: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.^d: 0.000075 riboflavin: 0.0010 ascorbic acid.

^b All systems dried from the frozen state under vacuum.

^c Calcium phospho-caseinate.

^d Fat globule "membrane".

reduction capacity and the effect of heat, thus confirming the data of figure 5. Serum protein also contributed significantly to reducing capacity and to heat susceptibility, and the materials of the fat globule "membrane" made a small contribution also.

Production of reducing substances during storage. The formation of acid-ferricyanide reducing substances during storage of simplified systems dried from the frozen state was studied. Samples of each system were stored under nitrogen over 45 or 60 per cent sulfuric acid at 37 or 50° C. for varying periods of time. Samples of spray dried whole milk and of whole milk dried from the frozen state were included for comparison. The 45 and 60 per cent sulfuric acid solutions furnished vapor pressures at 37° C. comparable to those that had been found to be in equilibrium with dry whole milk containing 5.4 and 2.7 per cent moisture,

TABLE 4
Reducing capacity of frozen-dried simplified systems and effect of storage thereon

System no.	Constituents ^a	Acid ferricyanide reducing substances as cysteine-HCl per g. complete system									
		Fresh			90 days at 37° C.				30 days at 50° C.		
					Over 60% H ₂ SO ₄				Over 60% H ₂ SO ₄		
		A ^b	B	C	Over 45% H ₂ SO ₄		Over 60% H ₂ SO ₄		Over 60% H ₂ SO ₄		60 days at 50° C.
		(mg.)	(mg.)	(mg.)	A	B	A	B	A	B	C ^c
1	Caseinate				(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
2	1 + lactose	0.15	0.17	0.05	4.95	4.90	0.41	0.43	1.11	0.63	0.08
3	2 + serum prot.	0.22	0.28	0.20	6.47	6.03	0.72	0.67	1.90	0.99	1.90
4	2 + milk fat	0.20	0.21	0.20	5.58	4.98	0.60	0.55	1.10	0.91	1.90
5	4 + f.g.m. ^d	0.19	0.25	0.25	5.50	4.84	0.77	0.56	1.60	0.84	2.72
6	5 + serum prot.	0.32	0.38	0.39	7.46	5.86	0.97	0.70	2.41	1.24	1.77
7	6 + riboflavin	0.22	0.35	0.33	7.85	5.36	0.92	0.72	2.25	1.26	1.72
8	7 + ascorbic	0.49	0.53	0.50	8.16	5.97	1.08	0.84	2.05	1.62	1.99
9	Whole milk:										
	Frozen dried		1.22		10.28		2.05		3.00		
10	Spray dried		1.63		8.81		2.33		3.07		

^a Ratio of constituents was as follows: 1.00 casein: 2.15 lactose hydrate: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.^d: 0.000075 riboflavin: 0.0010 ascorbic acid.

^b Letters designate replicate series.

^c Stored 34 days.

^d Fat globule "membrane".

respectively. The quantities of simplified systems prepared, particularly those containing serum protein, were insufficient to permit satisfactory moisture determinations by the toluene distillation method.

Here again the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of complete system. The data, recorded in table 4 show that even the most complete system employed failed to exhibit a reducing capacity of over half of that of dry whole milk. There was only a slight increase in reducing capacity of the caseinate system during storage. Interaction of caseinate and lactose was responsible for the major portion of the increase produced in storage. Serum protein also contributed materially to the original- and storage-produced reducing

TABLE 5
*Non-protein reducing capacity of frozen-dried simplified systems
and effect of storage thereon*

System no.	Constituents	Acid ferricyanide reducing substances as cysteine-HCl per g. complete system							
		Fresh		90 days at 37° C.				30 days at 50° C.	
				Over 45% H ₂ SO ₄		Over 60% H ₂ SO ₄		Over 60% H ₂ SO ₄	
		A	B	A	B	A	B	A	B
		(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
2	Caseinate + lactose	0.03	0.05	0.65	0.45	0.12	0.14	0.13	0.10
3	2 + serum prot.	0.05	0.09	1.00	0.62	0.22	0.17	0.20	0.22
4	2 + milk fat	0.06	0.04	0.68	0.37	0.27	0.15	0.08	0.16
5	4 + f.g.m.	0.07	0.09	0.53	0.32	0.12	0.15	0.15	0.21
6	5 + serum prot.	0.08	0.08	0.65	0.41	0.20	0.13	0.35	0.16
7	6 + riboflavin	0.08	0.06	0.85	0.41	0.27	0.16	0.35	0.22
8	7 + ascorbic	0.35	0.21	0.94	0.57	0.35	0.19	0.50	0.33
9	Whole milk: Frozen-dried	0.71		2.41		0.77		0.99	
10	Spray-dried	0.68		1.70		0.77		1.04	

capacities. The effect of the fat globule "membrane" materials was small and variable. Milk fat and riboflavin were inert but, as expected, ascorbic acid definitely enhanced the reducing capacity.

The data in table 5 indicate that ascorbic acid is the major non-protein reductant of acid ferricyanide in the fresh systems, but that non-protein reducing materials are produced upon storage by reactions involving the proteins or lactose or both. As was the case with total reducing capacity, the most complete simplified system failed to account for all of the non-protein reducing capacity of dry whole milk.

DISCUSSION

The modified ferricyanide method in which the pH is raised to 6.6 and the temperature lowered to 50° C. appears to yield as satisfactory re-

sults as that originally described by Chapman and McFarlane. It has the advantage of eliminating formation of a blue precipitate during the heating.

Ascorbic acid has been found to reduce ferricyanide stoichiometrically under the conditions adopted for the reaction. The amount of ascorbic acid present in milk, however, accounts for only a fraction of the ferricyanide-reducing capacity. Thus, for a milk containing 20 mg. of reduced ascorbic acid per l. (125 g. of solids), it may be calculated from the standardization curves that ascorbic acid would account for a reducing capacity equivalent to 0.36 mg. cysteine hydrochloride per g. of solids out of a total of about 1.00 mg. per g. Actually the effect of addition of ascorbic acid in the amount of 25 mg. per liter to artificial systems was somewhat less than this, amounting to the equivalent of 0.27, 0.18 and 0.17 mg. cysteine hydrochloride per g. in the three series reported in table 4. Obviously, considerable differences in the acid ferricyanide reducing capacity of milk may result from variation in the degree of oxidation of its ascorbic acid content.

Such relatively simple sulfhydryl compounds as cysteine and glutathione are also effective reductants of ferricyanide under the conditions used. According to Brand and Kassell (4) the cysteine content of β -lactoglobulin is about 1.10 per cent (analyzed after acid hydrolysis). If this figure be assumed to apply to the entire 0.70 per cent serum protein of milk, and if the sulfhydryl groups of milk proteins were as reactive as those of cysteine or glutathione, the protein sulfhydryls of milk would furnish a reducing capacity equivalent to about 0.80 mg. cysteine hydrochloride per g. of solids. Actually, the fact that the contribution of milk serum protein falls far short of this value (see table 4) constitutes evidence that the sulfhydryls of protein are much less active than those of the simpler compounds.

The failure of the most complete simplified system prepared to exhibit more than one-half the reducing capacity of fresh or frozen-dried whole milk could conceivably be due to absence of some milk reducing system from the simplified preparation. On the other hand, the reducing capacity of one or more of the constituents of the simplified system might possibly have been altered in isolation and purification. Such an effect would be most probable with the serum protein. A third possibility is that the environment of the reducing materials in the simplified systems is different enough from that in milk to account for the difference. The results reported in this paper give no clue to the reason for the discrepancy.

In spite of the failure quantitatively to duplicate the reducing capacity of fresh whole milk, the simplified systems do exhibit increases in reducing capacity upon heat treatment and storage which are quite comparable to those observed with milk itself. Furthermore, they indicate that these in-

creases are due to materials formed in part by reactions of the proteins with lactose and in part by reactions involving lactose in the presence of buffer salts.

The method is being applied to further study of factors influencing the changes occurring in dry whole milk during storage.

SUMMARY AND CONCLUSIONS

A modification of Chapman and McFarlane's ferricyanide procedure for evaluating the reducing capacity of milk is presented. This modification, which involves raising the pH to 6.6 and lowering the temperature to 50° C., proved somewhat more satisfactory, particularly with milk powders of high reducing capacity, than the original method. The method has been calibrated in terms both of ferricyanide reduced and of cysteine or ascorbic acid oxidized.

The capacity of milk to reduce ferricyanide is increased both by heat treatment and by spray drying. Study of simplified systems of milk constituents has shown that some of the reducing substances produced by heat treatment of milk and aging of dry milk are formed from lactose and from protein-lactose interactions.

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THE INFLUENCE OF *MYCOTORULA LIPOLYTICA* LIPASE UPON THE RIPENING OF BLUE CHEESE MADE FROM PASTEURIZED HOMOGENIZED MILK¹

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The present trend in the dairy industry is to manufacture cheese from pasteurized milk, both for public health reasons and to aid in the control of microbial defects. When blue cheese is made from pasteurized milk the product does not develop a full and typical flavor during ripening (6, 10); this has been attributed primarily to the inactivation of milk lipase by pasteurization (10), resulting in less hydrolysis of the butterfat of the cheese. The solution of this problem seems to lie in the substitution of a suitable lipolytic enzyme for the milk lipase inactivated by pasteurization. The present study was undertaken to explore the possibility of substituting the cell-free lipase produced by *Mycotorula lipolytica* (15, 16) for normal milk lipase in the manufacture of blue cheese from pasteurized homogenized milk.

HISTORICAL

Methods for the manufacture of blue cheese from raw cows' milk have been described by different workers (6, 12, 18). Lane and Hammer (9) modified the procedure formerly used by homogenizing the raw milk. This modification resulted in faster ripening of the cheese as well as in more luxurious mold growth, as compared with similar cheese made from nonhomogenized milk. Later the same workers (10) reported that blue cheese made from pasteurized homogenized milk was a more satisfactory product than that made from raw, nonhomogenized milk, but less satisfactory than if raw homogenized milk was used. They also observed that milk lipase definitely aided in the ripening of blue cheese. Fabricius and Nielsen (5) were able to produce a satisfactory blue cheese from raw, nonhomogenized milk by using a combination of heat and vacuum treatment of milk, namely 165–175° F. and 19 inches of vacuum. This treatment destroyed most of the undesirable microorganisms present in the raw milk without inactivating the milk lipase.

Irvine (8) added a commercial lipase preparation, later reported as steapsin (13), at the rate of 0.5 and 1.0 g. per 100 lb. of raw milk; the addition of the enzyme preparation resulted in accelerated fat hydrolysis and quicker ripening of the cheese as compared with the control, but a bitter flavor resulted in the cheese. Similar results were obtained by Coul-

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ter and Combs (3), who also used steapsin to hasten the ripening of blue cheese made from raw nonhomogenized milk. Thibodeau and Macy (17) added the enzymes of *Penicillium roqueforti* in the form of mycelium at the time blue cheese made from raw milk was hooped. The addition of 6 g. of mycelium to 5 lb. of cheese reduced the curing period from 10 to 5 months total time, as compared with the control without added mycelium. Parmelee (14) added pure cultures of *Alcaligenes lipolyticus*, *Achromobacter lipolyticum*, *Pseudomonas fragi* and *Mycotorula lipolytica* separately to lots of pasteurized homogenized milk made into blue cheese, and found that of these microorganisms only certain strains of *M. lipolytica* improved the flavor score and increased significantly the total volatile acidity of the cheese. This investigator also added to pasteurized homogenized milk special cultures of *P. roqueforti* grown on a modification of Czapek's medium containing 10 per cent butterfat and obtained cheese which were much superior to those made without the added special culture.

METHOD

Regular pasteurized homogenized milk of 3.5-3.8 per cent butterfat content was used in all experiments in quantities of 105 to 110 lb. per vat or lot. Three or four vats were used at one time, comprising a series, and conditions were kept as uniform as possible throughout the manufacture of cheese in each series. The vat contents were kept at 90° F. from the time of adding the culture until the curd was hooped. One per cent starter was used, and rennet was added at the rate of 90 ml. per 1,000 lb. of milk after 30 minutes holding time in series 1 to 4, and after 60 minutes holding time in series 5 to 7. Calcium chloride was added at the rate of 0.015 per cent (7 g. per 100 lb. of milk) to the milk in series 3 to 7 prior to setting. At the same time a previously standardized cell-free lipase preparation from *M. lipolytica* (16) was added in definite quantities to all but the control lot of milk in each series. The curd was cut into 0.5 inch cubes 70 minutes after setting and held for 2 hours with some stirring every 30 minutes, after which time the whey was drained. One per cent salt and 0.01 per cent mold powder were added to the curd at the time of hooping. The cheese was dry-salted daily for 4 days, using a total of 5 lb. of salt per 100 lb. of curd. Next the cheese was skewered and placed in the ripening room at approximately 10° C. and a relative humidity of approximately 90 per cent, where it remained for 12 weeks.

The cheese were examined and scored for positive flavor, defects, and visual mold growth after ripening periods of 4 and 12 weeks. A score of 10 was considered perfect in each of the three items under consideration. The total volatile acidity of the cheese was determined by the method of Lane and Hammer (10) at the ages of 4 and 12 weeks. Determinations of moisture, fat and total chlorides in the cheese at 4 weeks showed only

TABLE 1

*Preliminary trials on the influence of the addition of various amounts of *M. lipolytica* lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese*

Lot no.	Amount of lipase added ^a	Vol. acidity in ml. 0.1 N acid per 200 g. of cheese			Score								Remarks on flavor at 12 weeks
					Flavor				Mold				
					Positive		Negative						
					4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	
Series 1													
11	None	6.0	18	b	-	-	-	-	-	-	-	-	Lacking, slightly unclean
12	100	6.6	18	-	-	-	-	-	-	-	-	-	Lacking, slightly unclean
13	200	7.0	16	-	-	-	-	-	-	-	-	-	Lacking, sl. unclean, bitter
Series 2													
21	None	5.4	13	-	-	-	-	-	-	-	-	-	Lacking, sl. unclean
22	300	9.6	22	-	-	-	-	-	-	-	-	-	Sl. soapy, sl. unclean
23	800	18.0	65	-	-	-	-	-	-	-	-	-	Sl. sharp, soapy
Series 3													
31	None	7.0	13	3.0	4.0	4.0	3.5	3.5	4.0	3.5	4.0	3.5	Lacking, sour
32	600	26.0	50	7.0	7.5	6.5	7.0	4.0	7.0	4.0	7.0	4.0	Fair, sl. sharp, sl. soapy, sl. sour
33	1300	39.0	85	6.5	6.0	5.5	3.0	3.5	5.0	3.5	5.0	3.5	Excessively sharp and soapy
Series 4													
41	None	9.0	18	3.5	4.0	6.0	3.5	7.5	7.5	3.5	7.5	3.5	Lacking, sl. nutty, sl. fermented
42	300	15.0	38	6.5	5.0	6.0	4.0	3.5	7.0	3.5	7.0	3.5	Fair, sl. nutty, sl. unclean
43	600	21.0	60	8.0	7.5	8.0	7.0	5.5	4.5	3.5	4.5	3.5	Sl. sharp, sl. soapy
44	900	26.0	72	7.0	5.5	5.5	3.5	3.5	6.0	3.5	6.0	3.5	Excessively sharp and soapy

^a Calculated as total lipase activity (ml. of preparation \times lipase activity per ml.), the activity being expressed in acid degrees, which are defined as ml. of N NaOH required to neutralize the free fatty acids in 100 g. of fat (1).

^b No numerical score given.

TABLE 2

Further trials on the influence of the addition of various amounts of M. lipolytica lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese

Lot no.	Amount of lipase added*	Vol. acidity in ml. 0.1 N acid per 200 g. of cheese				Score								Remarks on flavor at 12 weeks
						Flavor				Mold				
						Positive		Negative						
4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks			
Series 5														
51	None	6.5	20.0	4.0	4.0	3.0	4.0	6.0	6.5	Lacking, musty, unclean				
52	250	10.5	27.4	5.0	4.5	4.5	5.5	6.0	2.5	Lacking, musty, sl. unclean				
53	375	10.5	33.7	5.5	6.0	6.0	5.0	5.5	5.0	Sl. lacking, sour, sl. unclean				
54	500	12.0	37.4	6.0	7.0	6.0	7.0	5.0	6.5	Fair, sl. sour, sl. unclean				
Series 6														
61	None	8.0	21.6	3.0	4.0	1.0	3.5	6.0	4.5	Lacking, bitter, musty, sl. sour				
62	250	11.0	31.2	6.0	4.5	6.0	4.5	5.0	4.5	Lacking, bitter, musty, sour				
63	375	11.0	34.6	5.5	6.0	5.0	7.0	6.0	5.0	Sl. lacking, sl. sour, sl. unclean				
64	500	13.0	35.4	6.5	6.5	6.0	6.0	5.0	7.0	Fair, sour, nutty				
Series 7														
71	None	7.0	19.5	4.0	4.5	3.5	5.0	6.0	6.0	Lacking, musty, sl. sour, fermented				
72	250	9.0	29.3	5.0	6.5	5.0	7.5	7.5	6.5	Lacking, sl. sour, sl. unnatural				
73	375	10.5	33.0	6.0	6.5	6.5	4.5	5.5	7.5	Sl. lacking, musty, unclean				
74	500	13.0	38.2	7.0	7.5	6.0	6.5	5.0	6.5	Fair, sharp, unnatural, sl. sour				

* Calculated as total lipase activity (ml. of preparation \times acid degree value per ml.) added to 105 lb. of milk.

slight variations within each series; these differences were not considered significant in the enzyme study under consideration, and therefore the data are not presented in this paper.

RESULTS

Data showing the influence of the addition of various amounts of *M. lipolytica* lipase upon the volatile acidity, flavor and mold growth of the cheese in series 1 to 4 are presented in table 1. The trials were of a preliminary nature and served to indicate the amount of enzyme required for the production of a blue cheese in which a satisfactory level of fat hydrolysis occurred. The control cheese (lots 11, 21, 31, 41) were lowest in total volatile acidity in their respective series and were lacking completely in the desired ketone flavor characteristic of properly ripened blue cheese. Additions of *M. lipolytica* lipase to the milk resulted in increases in the total volatile acidity of the cheese in proportion to the amount of lipase added. Cheese with total volatile acidity values of 50 and above at 12 weeks were criticized for being soapy and sharp, both characteristics being undesirable (lots 23, 32, 33, 43, 44). Lots 32 and 43 were most satisfactory from both body and flavor standpoint, although they also were criticized for being slightly soapy and slightly sharp.

Table 2 shows the results of replicate series 5, 6 and 7 made within a 5-day period after the complete data of the first four series had been collected. Again the total volatile acidity values of the controls (lots 51, 61, 71) were the lowest in each respective series, with the values increasing in the order of increasing enzyme concentration of the cheese. A close correlation existed between the total volatile acidity values of the cheese in the three series and the concentration of enzyme used. The flavor score, and to a certain extent also the defect score, showed good correlation with the total volatile acidity values, highest scores being given to lots 54, 64 and 74 which showed total volatile acidity values at 12 weeks of 37.4, 35.4 and 38.2, respectively. None of the cheese in these series was criticized for soapiness or excessive sharpness, although other defects were encountered; however, these could not be attributed to the enzyme added. This was also true in the first four series (table 1).

There was no indication in the cheese of any one of the seven series that mold growth was affected by the different amounts of total volatile acidity present at any time in the individual lot of cheese. No correlation could be established between mold score and flavor score of any one cheese. Although the mold scores of the different cheese varied from 4 to 7.5, all of the cheese showed sufficient mold growth to permit flavor development if other conditions were satisfactory.

DISCUSSION

The addition of *M. lipolytica* lipase to pasteurized milk which then was made into blue cheese brought about the desired hydrolysis of the fat.

The acidity of the cheese and the temperature at which the cheese was ripened both were favorable for the action of the lipase, as had been anticipated from previous study of this enzyme system (16). A good relationship existed between the amount of enzyme added and the values for total free volatile fatty acid obtained at 4 and 12 weeks of ripening of the cheese. The cheese containing the added lipase had more organoleptically detectable free fatty acids, as well as ketone flavor, and a waxier body than the control cheese without added lipase. These observations suggest that the lipase added was of considerable value in aiding in the proper ripening of blue cheese. According to Lane and Hammer (10), a satisfactory ripened blue cheese was not obtained until after 16 weeks' holding time, when pasteurized, homogenized milk was used, while with raw homogenized milk a satisfactory ripened cheese was obtained in 12 weeks. Thus the presence of lipase, either milk lipase or added lipase such as used in this study, brings about early hydrolysis of the fat and thus enables the mold to utilize the free fatty acids and to change certain ones into flavor-producing ketones (7).

The sharp, soapy taste in a number of cheese was correlated with free fatty acid values of 50 and higher in the cheese after ripening for 12 weeks. Cheese with most desirable flavor at this age showed values between 30 and 50. Other workers have made observations which support this conclusion (10, 14). The flavor of the cheese containing the added lipase, while characteristic of blue cheese, did not duplicate exactly the flavor of the product made from homogenized raw milk. However, most of those who sampled the cheese made from pasteurized homogenized milk containing the added lipase accepted the product as satisfactory cheese with a high level of good flavor development. Under no circumstances was a bitter or other objectionable flavor definitely attributable to the addition of the microbial lipase. Less breakdown of the body to a desirable level was observed in the controls than in the cheese made with added lipase. Since *M. lipolytica* is both lipolytic and proteolytic, it is possible that the cell-free lipase preparation also carried some proteolytic enzymes which were beneficial to the breakdown of the protein in the cheese. No data were collected on this phase of cheese ripening, although a study of this point would be desirable.

The repeatedly observed close relationship between total activity of lipolytic enzyme preparation added to the milk and extent of fat degradation in the resulting cheese permitted the addition of predetermined amounts of enzyme which would result in the desired level of total volatile acidity in the cheese after the ripening period of 12 weeks employed in this study.

The data indicate that cell-free lipase obtained from cultures of *M. lipolytica* could be used to advantage in the manufacture of blue cheese

made from pasteurized homogenized milk, and possibly also in other varieties of cheese in which hydrolysis of fat is essential for the proper ripening of the cheese.

SUMMARY AND CONCLUSIONS

1. Seven series of blue cheese were made from pasteurized homogenized milk with and without the addition of a cell-free lipase preparation obtained from *Mycotorula lipolytica*.

2. Examinations of the cheese at 12 weeks for flavor and other desirable characteristics showed the cheese ripened with the aid of the cell-free lipase preparation consistently was more satisfactory than the corresponding control containing no added lipase.

3. Increases in the concentration of lipase in the cheese resulted in increases in total volatile acidity values of the cheese and also of the intensity of the flavor typical of blue cheese. Cheese with enzyme concentrations high enough to show total volatile acidity values of from 30 to 50 after ripening for 12 weeks were most satisfactory in flavor. Cheese with total volatile acidity values above 50 were criticized as being sharp and soapy in every case.

4. The results of this study indicate that the cell-free lipase prepared from cultures of *M. lipolytica* can be used advantageously in the ripening of blue cheese made from pasteurized homogenized milk.

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MOISTURE STUDIES IN DRY PRODUCTS OF MILK. II. ESTIMATING WATER OF CRYSTALLIZATION OF ALPHA-LACTOSE IN DRY WHEY SOLIDS

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In the previous paper on the kinetics of moisture desorption of crystalline *alpha*-lactose hydrate (1), a possible method was suggested for estimating the water of crystallization of lactose in dry products of milk. In the present communication experimental evidence in support of the method, together with results obtained on dry whey solids, is presented. Dry whey solids have been selected for study, because samples with the lactose largely in the form of the crystalline hydrate are readily available.

The lactose in nonfat dry milk solids and dry whey solids manufactured by the ordinary spray and roller processes has been reported to be amorphous (5, 7). In recent years, however, various processes have been developed for inducing crystallization of lactose as the *beta*-anhydride or as the *alpha*-hydrate in dry whey solids (5).

Sharp *et al.* (6) have found that the state of lactose in dry products of milk has a great influence on the determination of moisture by the toluene distillation method. For products containing crystalline lactose hydrate, a longer distillation is necessary than for similar products in which the lactose is in the amorphous state. Presumably, the loss of moisture at the later stage is due to the dehydration of crystalline *alpha*-lactose hydrate. In a previous study on crystalline *alpha*-lactose hydrate in boiling toluene (1), this laboratory observed that the rate of dehydration follows the first order kinetics expression,

$$k = \frac{2.303}{t} \log \frac{a}{(a-x)}$$

where k is the rate constant, a the initial moisture content, and x the amount of moisture removed in time t . Therefore, it appears possible to estimate the water of crystallization of *alpha*-lactose and consequently crystalline lactose hydrate by taking advantage of this difference in the rates of moisture removal and of the unimolecular character of the dehydration of crystalline lactose hydrate.

EXPERIMENTAL PROCEDURE

Moisture desorption method. The apparatus used was exactly the same as that employed previously in the study on crystalline *alpha*-lactose hydrate (1). Fifty grams of sample were weighed into the 300-ml. Erlenmeyer flask and quickly covered with 100 ml. of moisture-free toluene. After at-

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taching the flask containing the sample to the apparatus, toluene was added through the top of the condenser to fill the moisture trap. Stirring then was applied to keep the mixture well agitated. The rate of distillation was adjusted to give more than two drops per second (1). At 5- or 10-minute intervals after the first appearance of moisture in the trap, the volume of water collected was read and multiplied by two to convert to per cent of

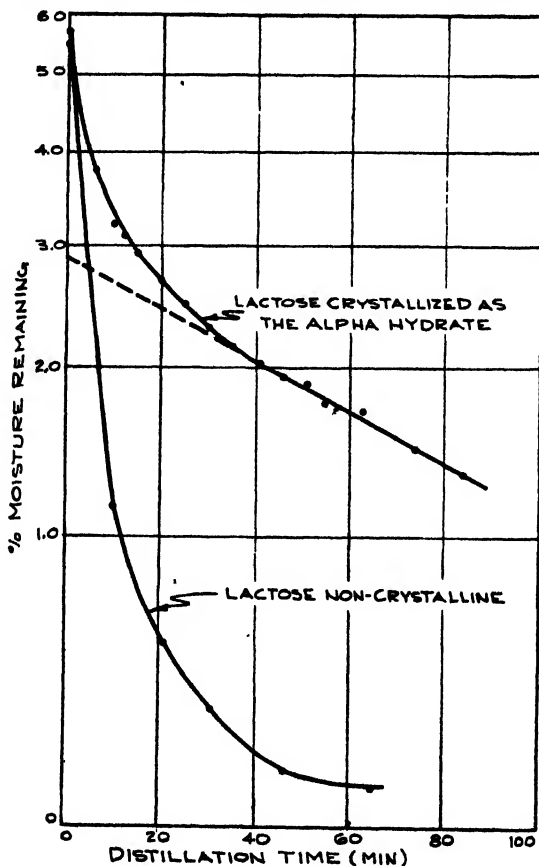


Fig. 1. Typical moisture desorption curves of two different types of dry whey solids.

water desorbed. The total moisture was determined by the Karl Fischer method (2). Less accurately, it may be estimated by continued distillation until a sufficiently constant value is obtained; this generally requires about 3 hours for the type of dry whey solids studied. The logarithm of the per cent moisture remaining in the sample, *i.e.*, $\log(a-x)$, at each time interval was plotted against the distillation time in minutes. The top curve (fig. 1) has two portions. The initial steep portion represents the dehydration of

crystalline *alpha*-lactose hydrate together with the protein hydrates and surface moisture. The second portion is linear and represents the dehydration of crystalline *alpha*-lactose hydrate. Consequently, by extrapolating the straight-line portion to zero time, the initial percentage of water from crystalline *alpha*-lactose hydrate may be obtained by taking the antilogarithm of the vertical intercept. If desired, the percentage of crystalline *alpha*-lactose hydrate present in each sample can be obtained by dividing the determined per cent of water of crystallization by 0.050.

Indirect method. The indirect method referred to in table 1 is a combination of two determinations: (a) total moisture by the Karl Fischer pro-

TABLE 1
Water of crystallization of alpha-lactose in some dry whey solids by two methods

Sample no.	Indirect method			Desorption method
	% Total H ₂ O (Karl Fischer)	% Free H ₂ O (vac. oven)	% H ₂ O crystallization	
1	5.68	2.73	2.95	(%) 2.89
2	5.31	2.55	2.76	2.70
3	5.44	2.82	2.62	2.73
4	5.27	2.48	2.79	2.89
5	5.79	3.08	2.71	2.52
6	4.47	1.89	2.58	2.60
7	5.43	2.68	2.75	2.67
8	4.91	2.00	2.91	
9	3.88	1.23	2.65	2.69
10	5.76	3.10	2.66	2.95
11	4.21	1.71	2.50	2.62
12	3.81	1.38	2.43	2.40
13	4.33	1.84	2.49	2.51
14	3.70	1.30	2.40	2.45
15	3.67	0.87	2.80	2.88
16	4.93	2.13	2.80	2.94

cedure of Fosnot and Haman (2) using visual end-point estimation and (b) "free" moisture by dehydration of a 4-g. sample in a Cenco-DeKhotinsky vacuum oven at 65° C. and 2-3 mm. mercury pressure for 5 hours (4). This method again is based upon the fact that lactose hydrate dehydrates at an extremely slow rate under the conditions used in the determination of "free" water. Thus, in two experiments with crystalline lactose hydrate of particle sizes less than 149 μ , only 0.05 and 0.07 per cent of moisture were removed in 5 hours. On the other hand, dry casein containing approximately 8 per cent moisture appeared to be completely dehydrated. The difference between the Karl Fischer result and that obtained in the low temperature vacuum oven determination was inferred to be water of crystallization of *alpha*-lactose.

Samples. All samples of dry whey solids used in this study were selected from samples currently sent to this laboratory for analysis. The particle

sizes of these products generally are within the range of about 54 to 210 μ . Crystalline *alpha*-lactose hydrate was Baker's C.P. powder containing the theoretical 5.0 per cent water of crystallization as determined by the Karl Fischer method (2). The particle sizes were under 149 μ . Dry casein was of technical grade obtained from J. T. Baker Chemical Company.

RESULTS

Figure 1 shows two typical moisture desorption curves for two different types of dry whey solids. For the top curve the lactose in the product is

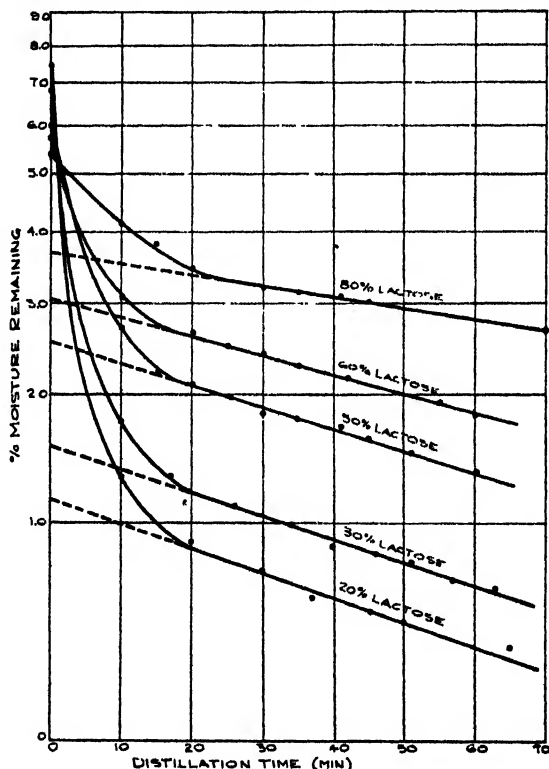


Fig. 2. Dehydration curves of mixtures of *alpha*-lactose hydrate and casein.

present largely in the form of the crystalline *alpha* hydrate, as indicated by the seeding test used by Troy and Sharp (7). This curve illustrates the initial rapid loss of moisture and the slower constant desorption after the first 20-30 minutes. For the lower curve the lactose is in the glass or amorphous state, as shown by a negative seeding test. This type of dry whey solids forms a single hard mass in boiling toluene and, in spite of the resultant reduction of surface area, shows a rapid rate of dehydration.

Since protein and lactose are the two major constituents in most dry products of milk, the method was applied to mixtures containing different proportions of dry casein and lactose hydrate to see how well the latter can be recovered. Results are plotted in figure 2. The casein used for the first two trials contained approximately 7 per cent moisture and was less than $149\ \mu$ in particle size. The remaining trials were conducted with dry casein of slightly higher moisture content and of particle size less than $210\ \mu$.

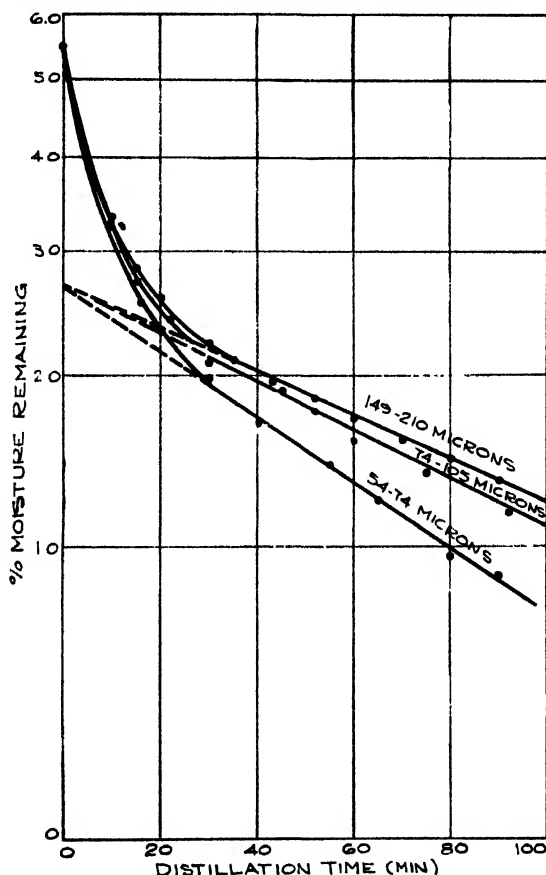


FIG. 3. Effect of particle size on the determination of *alpha*-lactose hydrate in dry whey solids.

A comparison of results by the desorption method with those by the indirect method on samples of dry whey solids which gave positive seeding tests for crystalline *alpha*-lactose hydrate is presented in table 1. Under conditions employed in the vacuum oven test for "free" moisture, weight loss after 5 hours of heating was constant.

In order to determine whether particle size has any effect on the final result, a sample of dry whey solids was fractionated into different particle sizes by means of standard sieves. Results are plotted in figure 3. The straight lines used for extrapolation were calculated by the method of least squares.

DISCUSSION

The difference in moisture desorption behavior of the two types of dry whey solids is quite evident from figure 1. It can be seen that the presence of crystalline *alpha*-lactose hydrate gives rise to a slower rate of desorption, which is unimolecular at the later stage. Since the composition of the two types of dry whey solids is approximately the same, it is unlikely that this difference in the rate of desorption could have arisen from any other sources.

The results of the experiments using crystalline *alpha*-lactose hydrate and dry casein, as shown in figure 2, indicate that at least in simple mixtures of the two materials, water of crystallization of lactose can be quantitatively differentiated from water adsorbed by casein. Moreover, the similarity of curve 1 shown in figure 1 to those in figure 2 tends to support the previous interpretation of each portion of the curve.

The apparent agreement between results obtained by the moisture desorption method and the indirect method as shown in table 1, in all probability, is not accidental. Admittedly, both methods are based upon the slowness in the dehydration of crystalline *alpha*-hydrate as compared with other moisture adsorbing constituents. Yet the two methods differ entirely in other respects. Whereas one method determines water of crystallization of lactose hydrate from the difference between total and "free" moisture, the other depends upon the unimolecular character of the dehydration of crystalline lactose hydrate in boiling toluene. Agreement between the two series of results must be considered good in view of the fact that the moisture determinations by even the best available methods usually involve deviations of the magnitude of 0.1–0.2 per cent.

Referring to figure 3, particle size within the range studied does not seem to have any influence on the extrapolated value in the desorption method aside from changing the rate of dehydration. Ideally, a sample should be homogeneous with respect to particle size. Practically, it has been found that for particle size occurring normally in dry whey solids of the type studied, the linear relationship for the dehydration of crystalline *alpha*-lactose hydrate still is obeyed.

From the above evidence it appears that both the moisture desorption method and the indirect method can be used for estimating water of crystallization of lactose and consequently of *alpha*-lactose hydrate itself in certain dry whey solids. Presumably, the methods can be applied to other dry products of milk containing crystalline lactose hydrate. It must be

pointed out that the desorption method depends on the continued presence of crystalline *alpha*-lactose hydrate after complete removal of all other forms of moisture. For this reason the method may not be as accurate for products containing small quantities of crystalline *alpha*-lactose hydrate as for the dry whey solids studied.

SUMMARY

A moisture desorption method has been developed for estimating the water of crystallization of *alpha*-lactose and indirectly the crystalline *alpha* hydrate itself in certain types of dry whey solids. It is based upon the difference in the rates of dehydration of crystalline *alpha*-lactose hydrate and other moisture adsorbing constituents and also upon the unimolecular dehydration of the hydrate itself.

Good recovery was obtained using mixtures of known composition of crystalline *alpha*-lactose hydrate and casein.

Results obtained by this method were in close agreement with those by an indirect method in which the difference between total moisture as determined by the Karl Fischer method and "free" moisture as determined by an oven procedure was considered to be water of crystallization of *alpha*-lactose.

Aside from an effect on the rate of dehydration, particle size, within the range of 54 to 210 μ , was found to have no influence on the results obtained by the moisture desorption method.

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THE NUTRITIVE VALUE OF HOMOGENIZED MILK: A REVIEW¹

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The consuming public seems to have accepted homogenized milk far beyond the anticipations of the early enthusiasts for the product. In the beginning, extravagant claims for the virtues of homogenized milk sometimes were made, mostly in good faith, to arouse interest in it. Once the consumer gave properly processed homogenized milk a fair trial, he generally became an enthusiast for the new product. In fact, homogenized milk once used often sold itself because of certain inherent qualities, and the extravagant claims were soon forgotten or ignored. Nevertheless, the belief persists in some sections that homogenized milk has superior nutritive values, thus presuming that nonhomogenized milk is lacking somewhat in those properties or values which give the full measure of nutrition. Consequently, the evaluation of the contributing factors affecting the nutritive value of homogenized milk in the light of scientific data on the subject seems desirable. Among the factors which may contribute to better nutrition when homogenized milk is used and which should be reviewed are: (I) palatability; (II) homogeneity; (III) curd tension; (IV) digestibility; and (V) miscellaneous factors. Also, it seemed expedient to review its use in infant feeding (VI) as well.

Extravaqant Claims for Homogenized Milk

The early attempts to introduce homogenized milk in America in the Province of Quebec, Canada, around 1909 were not successful. Nearly 20 years were to pass before the public accepted the product. It should be pointed out, however, that although at both periods the milks were called "homogenized," there was, in reality, a considerable difference between them. The homogenized milk introduced in 1909, rendered homogeneous by means of the Gaulin homogenizer, was sterilized in bottles at 226° F. for 20 minutes; that of 1927 was properly homogenized but was not a sterilized product. The homogenized milk of 1909 was heralded as a medicinal product, a "cure all"; that of 1927 was recognized merely as a milk of good flavor. For example, in 1909, Trudel (115) made the following thereapeutic claims for homogenized milk:

¹ Journal Article no. 944 (n.s.) from The Michigan Agricultural Experiment Station.

"'Laurentia' (homogenized) milk, especially the *maternized* (homogenized and sterilized)² constitutes a food of the highest order, and is particularly to be recommended for bottle babies, in cases of diseases of the digestive tube, such as gastroenteritis, green diarrhoea, cholera infantum and also for children suffering from ringworm of the scalp or face.

"The use of 'Laurentia' milk is also advisable for adults suffering from dyspepsia, acute or chronic gastritis, irritation of catarrh of the stomach consequent upon dietetic (*sic*) excesses or the *abuse* of alcoholic liquors. It also constitutes a valuable food for persons afflicted with kidney and liver diseases such as congestion of these organs, jaundice, cyrrhose (*sic*), acute or chronic neuritis (*sic*), Bright's disease, as well as in cases of inflammation of the bladder and the urinary canal. Finally, it is to be recommended in cases of infectious diseases, where the system already invaded by microbes and their secretions needs a perfectly aseptic food, under this heading we may include measles, scarlate (*sic*) fever, diphtheria, grippe, typhoid fever, etc., and also all kinds of poisoning, acute or chronic."

Thus, it is not surprising that early attempts to sell homogenized milk were not successful. Unfortunately, even after a lapse of over thirty years, some extravagant claims for homogenized milk still persist, so that Harding (52) in 1938 was impelled to write as follows:

"Homogenization has suffered more from its friends than from any other cause.

"They commonly say that homogenized milk digests much better than the untreated milk. As a matter of fact, milk is one of our most completely digested foods and much of this talk in favor of homogenization has been little less than a libel against the ordinary milk.

"It is hard to understand why some milk dealers will make such an unfair presentation regarding their ordinary milk in the hopes of selling a few bottles of homogenized milk. If the statements were true it would be a doubtful business procedure, and when there seems to be an entire lack of dependable data for the statement that homogenization makes any measurable increase in the digestibility of milk the business motive is hard to understand."

Advantages Reported for Homogenized Milk

Buttenberg (21), Pittius (88), Bordas *et al.* (16), Schoofs (92), Bishop and Murphy (13), and von Sobbe (94) early pointed out that the prevention of the removal of cream was one of the major advantages of homogenized milk. Sidersky (93) believed that the fresh milk taste of homogenized milk was a factor in its favor, as well as the stability of its fat emulsion. Istaz and Van Soest (64) stated that homogenized milk had a very fine flavor and was especially well suited for nutrition. Gaulin *et al.* (47) demonstrated to their own satisfaction that homogenized milk was "much more digestible" than nonhomogenized milk. In addition, Bishop and Murphy (13) reported claims that homogenized milk was more easily digestible.

More recently, Hess *et al.* (53) believed that the "greatest value of all these milk modifications (heat treatments and additions) may lie in the

² Reviewer's explanation.

soft, broken curd, which exposes a large surface to the digestion juices and to denaturization of the proteins." Jones (70) emphasized that when using homogenized milk every consumer would receive his fair share of milk fat since there could be no stealing of cream, leaving the skim milk for the children. He admonished, "Homogenized milk can never be skim milk." With wider use of homogenized milk, other nutritional advantages were claimed for it.

Wolman (127, 129) believed that "the nutritive benefit of adequately homogenized milk—whether pressure treated or sonized—rests in the small soft curds that form in the stomach of the individual who drinks the milk. Certain other advantages might be mentioned, namely, improved flavor, uniform distribution of the fat, and protection against theft of cream content." Later he (132) pointed out that the sanitary quality of homogenized milk was as important as its curd quality, stating that a low bacterial count could be maintained with higher-than-normal holder pasteurization temperatures without producing a detectable heated flavor.

Of 22 reasons classified by Tracy (105) from consumers as to why they used homogenized milk, 11 seemed to have a direct or indirect bearing upon its nutritive value. These were:

- a. "Tastes richer."
- b. "Easy to prepare for baby feeding."
- c. "The last glass is as good as the first."
- d. "No cream rises to top of glass standing in refrigerator."
- e. "There is no temptation to remove the cream."
- f. "There is no loss of cream in scum after boiling."
- g. "Feedings prepared for infants in advance do not have to be remixed when used."
- h. "Our youngsters refuse to drink any other kind of milk."
- i. "As a result of my children's drinking more of this milk, all have gained weight."
- j. "Nipples on bottles do not stop up and baby gets proper proportion of fat."
- k. "An infant born in October was fed prepared powdered milk but was unable to properly digest fat. Was then fed homogenized milk which has been digested easily and infant has been gaining weight steadily."

Wolman (131, 133) listed four advantages of homogenized milk of medical interest and importance when compared with ordinary market milks, namely: (a) uniform distribution of fat; (b) good flavor; (c) possibility of high sanitary quality; and (d) altered curd properties resulting in improved digestibility.

McLester (84) gave the following advantages for homogenized milk:

"It is more easily digested; because of the smaller size of the fat droplets to

which it is attached, the vitamin D is better utilized; the fat globules do not rise to the top but remained suspended, and the curd is smaller and more easily digested."

Another advantage of considerable merit is its suitability for infant feeding (33, 43, 46, 66, 69, 111, 127, 129, 130, 131), and in hospitals (33, 127).

A nutritional advantage of the use of homogenized milk in hospitals, institutions and restaurants is well stated by Doan (34) as follows:

"The process is of particular advantage where the milk is to be dispensed from bulk as is the case in hospitals, institutions, restaurants, etc. It will insure that each recipient of the product obtains a proper proportion of the fat and the 'cream' is not kept in the kitchen for benefit of the 'help'. In hospital use it is preferred by the dietitian because it is certain that each portion of the milk is identical and that one patient does not get 'light cream' and another skim milk."

The use of homogenized milk in schools would seem to be of a direct advantage. Kelly (71) found that when nonhomogenized milk was served to school children in half-pint bottles, an average of 5.6 per cent of the milk was left in the bottle. This remnant contained nearly 16 per cent of the fat. Obviously, the milk was not thoroughly mixed before serving; nor could the children be relied upon to do the mixing. Evidently when the straws were inserted, the bottom layer was drawn off first, while the creamy portion remained until the last. These data indicated that the school children were being deprived of a portion of the butterfat they were supposed to have. He concluded that this loss could be largely obviated by proper homogenization of all milk delivered to schools.

I. PALATABILITY

Palatability is an important attribute of foods, having an indirect bearing upon nutrition. Unless food is palatable it will not be readily accepted or ingested with anticipation. Moreover, it is reasonable to presume that larger quantities of palatable than of non-palatable foods will be consumed. Several factors, such as initial flavor, stability of flavor, homogeneity, appearance, smoothness, and absence of cream clots (93), influence the palatability of milk. Properly processed homogenized milk has those properties, but as pointed out by Hood and White (61), homogenized milk is more susceptible to an off-flavor due to light, the activated or sunshine flavor, than nonhomogenized milk.

That the early homogenized milk was not always palatable is not surprising. The early process involving sterilization of the milk yielded a cooked flavor. Furthermore, the role of lipase in decreasing the pH (32) and inducing lipolysis (42, 51) was not known. Despite the fact that increased acidities were encountered in early homogenization (16) and that much discussion was had in 1905 over a "bad" flavor, resembling butyric acid, which was believed to be due to the contamination and growth of specific bacteria in homogenized milk (47), Ladd (77) reported that homo-

genization decidedly improved the flavor of milk. However, Washburn and Jones (121) reported that homogenized milk was not as palatable as nonhomogenized milk. They observed that pigs apparently disliked homogenized milk. They noted that throughout the feeding trial the pigs which were fed homogenized milk were not inclined to gorge themselves and that the homogenized milk was not relished as well as the normal milk, although the animals to which it was fed had never been accustomed to cow's milk. It must be pointed out, however, that the milk was raw, was homogenized at 85° F., and was stored for some time—conditions favoring development of rancidity. Without a doubt this homogenized milk was so rancid and repulsive that even pigs drank it reluctantly. On the other hand, Berry (11) found that rats receiving homogenized milk consumed 17.5 ml. more milk per day per rat on the average than those having access to normal hard-curd milk. Tretsvén (108) found, also, that rats consumed more homogenized milk than normal hard-curd or normal soft-curd milk.

In comparing the flavor of nonhomogenized and homogenized milk, the flavors of the fresh products and those of samples stored for several days following processing should be considered. Homogenization does fix the clean, sweet flavor of milk against oxidation (107).

Babcock (6), securing 470 opinions on 470 samples each of homogenized and nonhomogenized milk as to its palatability, found that with milk of good flavor, properly homogenized, 62.1 per cent of the opinions showed no prejudice against homogenized milk. He (7) believed that the improved palatability reported for properly prepared homogenized milk might make it an important factor in increasing the per capita consumption of milk.

Trout (110) and Trout *et al.* (113), comparing the flavors of freshly processed products, noted that: (a) homogenization of pasteurized milk did not impair the flavor or cause the development of any undesirable flavor; (b) the flavor of homogenized pasteurized milk did not merit first choice over the nonhomogenized milk by the majority of the judges; (c) difficulty was encountered in picking out the homogenized samples from the nonhomogenized; and (d) the homogenized milk seemed smoother, and therefore, richer to some of the judges than the nonhomogenized pasteurized milk.

Doan (37), making a preference study, found that a greater number of people preferred homogenized to nonhomogenized milk at all fat levels from 2.5 to 5.0 per cent at 0.5 intervals but noted the highest percentage of preferences was obtained at the 4.0 per cent level. The consensus was that the process made the milk "richer", "smoother" or "creamier".

Homogenization retards or inhibits the development of the oxidized flavor of milk, as shown by Tracy *et al.* (107), by Thurston *et al.* (103), and later substantiated by Ross (89), Doan (35), Trout and Gould (112),

Larsen *et al.* (78), and Babcock (8). Thus, properly stored homogenized milk retains the fresh flavor over an extended period, a factor not to be discounted in evaluating the flavor of homogenized milk. Moreover, homogenized milk cannot be mixed or blended with raw milk and retain its palatability, for such mixtures soon become rancid (42, 49, 79).

Wolman (130) observed that homogenized cow's milk had a pleasing flavor. He (131) believed that the masking effect of homogenization on the possible "cooked" flavor when milk was pasteurized at 150° F., as shown by Spur (96), was beneficial in that the flavor was not impaired and the augmented pasteurization heat was of obvious importance as a sanitary safeguard.

· II. HOMOGENEITY

Properly processed homogenized milk retains its homogeneity over an extended period of time (93). This property early was recognized in the product. In fact, such milk originally was known as fixed milk, "lait fixe" (47), and the process itself was known as "fixation" (16, 92). That the product could not be creamed or churned in transport was the basis for the prediction of a favorable future for homogenized milk (93). Despite absence of cream-line formation on properly processed homogenized milk (106, 114), some upward migration of fat globules occurs upon long standing (40, 114), and some settling of the fat and solids-not-fat occurs when the milk is frozen and thawed slowly (110). Nevertheless, the actual upward migration of fat in a quart of milk is practically insignificant, slightly over 1 per cent, even when the United States Public Health Service maximum of 10 per cent differential between the percentage fat in the upper 100 ml. and the remainder of the quart has been reached. Thus, for all practical purposes, properly homogenized milk is virtually homogeneous throughout.

Distribution of vitamin A and vitamin D. Vitamins A and D, associated with milk fat, are distributed uniformly throughout properly homogenized milk and thus are available proportionately as the milk is consumed. There is no removal of the vitamin A- and D-bearing fat of homogenized milk in the form of cream for coffee, leaving the less-vitamin-rich milk for beverage use. Jeans (65) believed that when vitamin D was added to milk, its even distribution with the cream throughout the milk by means of homogenization was advantageous. Thus, homogenized milk appears to be the best possible vehicle for added vitamin D.

Marriott and Jeans (81) advised that in homogenized, vitamin-D milk the vitamin D was evenly distributed and, when the milk contained 400 units to the quart, the vitamin D intake of the baby was ample if customary amounts of milk were ingested. Krauss *et al.* (75), making bioassays by the line-test procedure of normal milk, homogenized milk and mineral

modified soft-curd milk, all from the same source, and natural soft-curd milk, all fortified to the extent of 400 units of vitamin D per quart, found the same degree of healing when equal amounts of each were fed. They concluded that the effectiveness of added vitamin D was not influenced by the curd-tension of the milk. McLester (84) believed that the vitamin D of homogenized milk was better utilized because of the smaller size of the fat globules to which it was attached.

III. CURD TENSION

There are many ways of lowering the curd-tension of cow's milk, such as acidifying, diluting, boiling, mineral modification, enzyme treatment and homogenization. Apparently, however, homogenized milk is the soft-curd milk most widely sold (132). In fact, homogenized milk often is advertised as soft-curd milk, thus featuring this property to the exclusion of other important factors bearing upon its nutritive properties.

As early as 1916 Washburn and Jones (121) noted that curds formed from homogenized milk were so much more flocculent and friable as a result of this process that these workers were led to believe that benefit might be expected from such treatment.

Not until 1923 when Hill (54) developed a test for determining the curd tension of milk, believing that the curd variance might be an index to the food value of milk for infants, was there much interest shown in soft-curd milk.

Since then many research workers (2, 10, 11, 22, 23, 33, 41, 75, 80, 86, 90, 102, 104, 105, 106, 113, 119, 120, 122, 123) have noted a lowering of the curd tension of milk as a result of the homogenization process.

Percentage reduction. Wallace (119) reported that homogenization reduced the curd tension about 50 per cent; Doan and Welch (41) found that proper homogenization reduced the curd tension to about 40 per cent of that of the original milk; while Theophilus, *et al.* (102) observed that homogenization at pressures of 500, 1,000 and 2,000 lb. reduced the curd tension of milk approximately 25, 46 and 53 per cent, respectively. Krauss *et al.* (75) noted that homogenization at 2,500 lb. pressure reduced the curd tension 61.5 per cent.

Essential pressures. Berry (11) concluded that homogenization pressures of 3,000 to 5,000 lb. were required to render hard-curd milk (50 to 112 g.) a soft-curd milk. The higher the curd tension of the original milk, the greater was the percentage reduction of curd tension after homogenization. Tracy (104, 106) showed that the curd tension of homogenized milk varied with the season and with the temperatures and pressures of homogenization and emphasized the importance of regulating the homogenizing processes so as to get as low curd tension as possible. He (105, 106) secured approximately maximum reduction in curd tension of milk

when homogenizing at 2,500 lb. pressure. Likewise, Caulfield and Martin (22), Doan (35), and Babcock (8) observed that pressures in excess of 2,500 lb. per square inch appeared to be of little practical value in further reducing the curd tension of milk.

Chambers (23) noted in sonic vibration studies that a direct relationship existed between the degree of fat dispersion and degree of curd tension reduction.

Jeans *et al.* (67) stated that homogenization gave soft-curd character to milk when the pressure and temperature were appropriate. Later Marriott and Jeans (81) advised that milk homogenized at pressures of 2,500 to 4,000 lb. to the square inch would have a curd tension less than 20 g.

Mechanism of the lowering of the curd tension of milk by homogenization. Lundstedt (80) attributed the curd softening effect of homogenization to the increased fat surface which "will remove up to 25% of the casein from the serum by the phenomenon of adsorption. The reduction in the concentration of the available casein results in a lowered curd tension." Evidently these statements are based upon the findings of Wiegner (124) to the effect that in the case of normal milk 2 per cent of the total casein was adsorbed on the surface of the fat globules, whereas with homogenized milk 25 per cent of the casein was adsorbed on the fat.

On the other hand, Sommer (95) explained that the effect of homogenization on curd tension was attributable to the increase in the number of globules serving as points of weakness in the coagulum and to a lesser extent to the casein adsorption on the increased fat surface area causing a lower concentration in the serum proper.

Nature of curd of homogenized milk. Not only is the curd tension of milk changed by homogenization, but its character is changed as well (116). Associated with the soft texture are smaller curds, thus increasing the total surface area (8, 130, 131).

Anthony (5), examining curds from homogenized milk retained in the human stomach 0.5 hour and then regurgitated, found them to be fine, uniform, soft, porous and permeable. Prior to this, however, Gaulin *et al.* (47) had reported with conviction as follows: "Fixed milk is much more digestible and I proved this by comparing homogenized milk with nonhomogenized milk. I placed in each vat a drop of the same rennet: the nonhomogenized milk gives a very thick curdled milk, hard as rubber; the other, on the contrary, a very divided curdled milk resembling the whites of eggs beaten like snow; obviously, this latter must be much more digestible than the former."^a

Curd-surface area. When the average curd tension of raw milk (46 g.) was reduced to 10 g. by homogenizing and pasteurizing, the average curd surface increased approximately 230 per cent (73). When a number of

^a Literal English translation from the original.

determinations were averaged, there appeared to be a rather definite inverse correlation between curd tension and curd area (74).

Storrs (100) observed that with untreated, homogenized, enzyme-treated, and base-exchange milks studied *in vitro* at pH ranging from 6.0 to 4.0, the amount of curd recovered from homogenized milk varied the least and tended to be somewhat bulkier than that of the other milks throughout the entire pH range. He (101) found no significant relationship between curd tension and curd surface area and believed that these characteristics were independent, each being influenced or determined, possibly, by factors not closely related.

However, Babcock (8) showed that, on the average, as the curd tension of milk was lowered by homogenization, the surface area of the curds increased, but the increases were not significant from the digestion standpoint until the milk was homogenized at a pressure of 2,000 lb. or more.

Spur and Wolman (99) found that the curd area (curd number or index) paralleled the curd tension; the homogenized milks, being soft-curd, yielded the higher curd numbers. Spur (98) noted that the curd numbers of commercial homogenized milks were considerably above 200 and consequently showed no large curds at all.

Soft curd of commercial homogenized market milk. The "softness" of the curd of commercial homogenized milk sometimes is questioned, with the inference that the milk is not properly homogenized with respect to pressure and temperature. The Council on Foods of the American Medical Association (2) pointed out that unless the conditions of processing were known to be suitable, the fact that the milk was homogenized was no assurance that the milk was soft curd.

Likewise, Chambers (24) tested homogenized milks in which there was quite satisfactory fat dispersion without any decrease whatever in curd tension, and without perceptible alteration in the curd size or texture. He (25) reported that "in many localities where several brands of homogenized milk are being sold on the same claim of enhanced digestibility, tests have shown that some actually show change in curd properties while others show no change whatever. These differences are usually traceable to improper maintenance of apparatus, inadequate homogenization pressure, improper temperature control, or other defects in plant operation over which there is at present no control."

Kugelmass (76) also warned that mere homogenization was no assurance that soft-curd milk had been produced unless the conditions of homogenization had been determined.

Nevertheless, Spur (97) found that the curd tension of grade A and grade B homogenized milks marketed by 36 dairies in Philadelphia averaged 11.6 and 11.2, respectively, and ranged from 5.3 to 18.4 g. and from

4.6 and 16.4 g., respectively. These values are well under either the 20 or the 30 g. standard.

IV. DIGESTIBILITY

As pointed out by Harding (52), many extravagant claims have been made for homogenized milk. Those probably recurring most frequently have a bearing upon its digestibility. Chambers (24) believed there had been altogether too much indiscriminate talk about the improved digestibility of homogenized milk, with the result that homogenization has become synonymous with ease of utilization. Such references as 'more completely digestible', 'more easily digested', 'more rapidly digested' and 'improved digestibility' appear to be more often the result of general observation and postulation rather than the result of controlled experimentation.

Chambers (24) believed that the design of satisfactory tests for digestibility of milk was difficult because of our incomplete knowledge of the sequence of events in the human digestive system. He pointed out that two possibilities existed, namely: (a) feeding the processed milk over a period of weeks or months to representative groups of normal children, and (b) chemical or physical tests of the milk which could be shown to correlate with the effect of feeding the product to infants. Of the latter, curd tension, curd size and *in vitro* digestion have been employed extensively.

Ease of digestibility of homogenized milk. Early speculation (4, 77) was made on the influence of homogenization on the digestibility of the milk, since the curd appeared soft and friable and seemed as though it should be penetrated more easily by the digestive juices. Gaulin *et al.* (47) were certain that "Le lait fixe est beaucoup plus digestible." Mayer (83) advised that homogenized milk was more easily digested than nonhomogenized milk and thus was good for sick patients.

Years later, Hiscox (58) stated that homogenization under proper conditions of temperature and pressure operates to reduce the curd tension of the milk, thereby rendering it more readily digestible.

Espe and Dye (45) believed that any factor that tended to make the digesting mass more porous, to lower curd tension or cause it to absorb gastric juice more rapidly, followed by peptonization and disintegration or liquefaction, shortened the digestive period. Working with natural soft-curd milk, they observed that doubling the curd tension of milk increased the length of the digestion period from 30 to 65 per cent.

Anthony (5) noted that curds from homogenized milk were porous and permeable, thus indicating easy admission of the digestants. Lundstedt (80) believed that homogenized milk was to be preferred to ordinary whole milk, not alone because it was more easily digested but because of its better taste. Hull (62), according to Babcock (8), reported digestion studies which showed that milk homogenized at 1,500 and 2,500 lb. pressure at a

temperature of 130° F. gave results almost the same as those obtained from regular milk.

However, Chambers *et al.* (27) concluded from their studies that the curd-surface area was an adequate index of the relative digestibility of milk in the infant's stomach. They demonstrated that some homogenized milk was improved in digestibility by processing to such an extent that it could be fed without further modification to premature and newborn infants.

Babcock (8) believed that the smaller curds of homogenized milk afforded a greater surface area for contact with the digestive juices, which would seem to make the milk more readily digestible than the nonhomogenized milk. McLester (84) stated the digestibility of milk could be enhanced by homogenization.

Speed of digestion of homogenized milk. Doan (36), in a critical review of the literature on soft-curd milk, stated that homogenized milk (including sonized) had not reacted very favorably in some *in vitro* studies, so that until further studies were made and particularly until careful clinical comparisons were available, little could be said in favor of soft curd milk. He (35) explained that homogenized milk did not exhibit as good digestibility characteristics as the curd tension would seem to indicate, apparently because the coagulum, although soft compared with unprocessed milk, was adhesive and held together in a mass instead of breaking up into a granular or flaky condition. Homogenization undoubtedly improved the digestibility of milk but the curd tension apparently was not an index of the improvement.

Later, Doan and Dizikes (38) found that the correlation between curd tension value and digestibility for more than 100 samples of a number of different types of milk, including homogenized, was rather poor. Their observation in part follows:

"While homogenized milk showed curd characteristics and digestion properties much superior to unhomogenized milk, particularly at the second and third hours, it was considerably inferior to acidified, superheated and evaporated milk and appreciably inferior to boiled milk. Sonized milk was slightly inferior to piston homogenized milk, as might be expected, since, in general, sonic vibrated milk is less effectively homogenized than milk treated with a piston machine."

Babcock (7, 8) found in *in vitro* studies that during the first 15 minutes of digestion 76.5 per cent and 56.5 per cent more digestion took place with the boiled and homogenized milks, respectively, than took place in the raw milk from which they were prepared. However, at the end of 2 hours, 15.4 per cent more digestion had taken place in the homogenized milk than in the raw milk, whereas only 10.3 per cent more digestion took place with the boiled milk than with the raw milk. At the end of 5 hours, digestion was practically the same for the raw and processed milk. These results indi-

cated that both boiled milk and homogenized milk were more quickly but not more completely digested than raw milk.

Kelly (73) reported that the relative rate of digestion of properly homogenized milk was similar to that of boiled milk.

Stomach-emptying time. Doan and Welch (41) observed that soft-curd milk appeared to be digested more rapidly and to be eliminated sooner from the stomach of humans, calves and rats than hard-curd milk. Examination of stomach contents revealed that the curd formed was different, the curds from milk of low tension being more friable and looser in makeup than those from milk of high tension.

Chambers and Wolman (26) found that when curd surface areas were compared with the usual curd tension, there was general agreement with the theory that curd tension is an index of the rate of gastric clearance, but exceptions did exist with those milks which were subject to drastic additions or subtractions.

Jeans *et al.* (67) stated that "Milk which produces a soft curd in the stomach leaves the stomach more quickly and is more readily digested than ordinary milk." Tretsven (108) noted that homogenized milk passed through the digestive tract of rats much faster than nonhomogenized milk. Wilcox (125) reported, as a result of roentgenological studies on adults, that homogenized milk left the stomach sooner than soft-curd and average milk.

Hadary *et al.* (50), using roentgenographic examinations of patients who ingested bariumized milks, concluded that no correlation existed between the curd tension of the bariumized milks and stomach or colonic emptying time of children and that soft-curd milks did not leave the digestive tract more rapidly than the hard-curd milks. They observed that at the 2-hour interval "the average per cent of stomach emptiness in the case of homogenized milk did appear to be significantly greater than for the other milks. However, this difference was not evident at four and five hours after feedings, all milks showing substantially similar degrees of stomach emptiness. Similarly, no statistically significant difference in elimination from the system was evident at twenty-four hours after feeding for the five test milks."

Wolman (135) concluded "in 72 'matched pair' experiments no measurable differences could be demonstrated in the intragastric responses to 'soft-curd' homogenized pasteurized milk as compared with the more 'hard-curd' plain pasteurized milk." The mean coagulation times of the ingested milks were 19.2 minutes for homogenized milk and 20.3 minutes for the pasteurized milk. Likewise, the mean emptying times were only one minute apart. The slightly more rapid stomach-emptying time when homogenized milk is ingested probably accounts for many of the statements concerning the improvements of the digestibility of homogenized milk.

Completeness of digestibility of homogenized milk. Ladd (77), in 1915, stated:

"Chevalier demonstrated by chemical analyses that the constituents of homogenized milk are more completely absorbed than those of simple sterilized milk. The more finely divided the food, the greater its accessibility to the digestive fluids, and the greater its assimilation. It is interesting to note also that when rennin is added to homogenized milk, the curd which results is a homogeneous flaky paste, resembling closely the curd of human milk."

Nevens and Shaw (87) from their studies concluded as follows:

"Many references are found in the literature of the 'ease of digestibility' of certain kinds of milk, and the term 'more digestible' is also commonly used. Many of these terms are deduced from the observations of physicians in cases in which they have found that one kind of milk agrees with the patient, while another kind causes more or less digestive disturbance or is unsatisfactory for some reason. There are many statements to the effect that evaporated milk and dried milk are 'more digestible' than fresh raw milk.

"The authors believe that their work helps to clarify the situation which now exists with respect to the term digestibility as applied to milk. Claims that homogenization, evaporation, or drying, or a combination of these factors, makes the protein and fat of milk more completely digestible, lack the support of adequate experimental evidence obtained in actual feeding tests. The author's findings, however, do not preclude the possibility that manufacturing processes such as those just mentioned may affect the time required for the digestion of the protein and fat, or that they may make the milk more readily tolerated by some individuals."

The Council on Foods of the American Medical Association (3), reviewing the literature on the digestibility of soft-curd milk, summarized as follows:

"There is evidence that a variety of milk preparations which yield soft-curds are well tolerated and well utilized by infants, children and older persons. In general, milk that has a low-curd tension as determined by appropriate laboratory methods leaves the stomach more quickly than milk that does not have this property. Such digestion as takes place in the stomach is more quickly accomplished when the curd is soft. The evidence is meager, however, that any soft-curd milk are 'better digested' or more completely digested than ordinary boiled milk."

Kelly (72) observed from some preliminary studies that homogenized milk was not only more rapidly but more completely digested than non-homogenized milk. Later he (74) noted that at the end of 5 hours the amount of proteolysis was practically the same for nonhomogenized and homogenized milks, although at 15 minutes 171 per cent more proteolysis took place with the homogenized milk than with the pasteurized milk. Babcock (8) concluded that boiled milk and homogenized milk were more readily but not more completely digested than raw milk.

Doan and Flora (39), after extensive *in vitro* studies on comparative digestibilities of several milks, concluded that curd tension value did not appear to be a reliable index of digestibility for all types of milk, particularly of homogenized milk. Homogenization lowered curd tension consid-

erably but apparently improved digestibility very slightly, if at all. They believed that curd-particle size apparently would be a more accurate index of the digestibility of milk and its suitability for use by infants than would curd tension. Likewise, Turner (116), using an *in vitro* digestibility test, found that homogenization did not alter appreciably the relative digestibility of cow's milk.

Digestion of homogenized fat. In the early days of milk homogenization, Birk (12) concluded that the reduction of the size of fat globules of cow's milk to that of human milk by homogenization possessed no advantage for well or sick infants over milk not so treated. However, Marriott and Schoenthal (82), in a study of the use of evaporated milk in preparation of infant formulas, predicted "Heating does not alter the chemical character of the fat of milk, but as evaporated milk is subject to a process of homogenization, the fat globules are broken up into much finer particles. Just how great a factor this size of the fat globules may be in rendering the fat more digestible is as yet undetermined. One might expect that the rapidity of digestion of homogenized fat would be greater due to the larger surfaces exposed to lipase action." Dorner and Widmer (42) noted that a number of specialists in pediatrics claimed that homogenized milk was dangerous for babies because it formed too much fatty acid in the intestine. Homogenization does accelerate the breaking down of the fat, but the question concerning the danger to infants has not been proved and Nevens and Shaw (87) pointed out that it is often claimed that manufacturing processes, such as homogenization, which reduce the fat globules to smaller size, increase the digestibility of the fat. However, results of their studies raised a question regarding the soundness of such claims, since the digestibility of the fat of fresh whole milk was so nearly complete that there was but little possibility of its being increased.

Likewise, Holt *et al.* (60) showed that the size of the "fat particles is without influence on fat absorption." Also, Marriott and Jeans (81) concluded that the breaking up of the fat globules in itself was of little importance in fat digestion.

V. MISCELLANEOUS FACTORS

Lack of distress from drinking homogenized milk. Brennemann (17) demonstrated experimentally with humans that "the casein of raw milk unless modified so that it will not form hard large coagula offers serious difficulties in digestion that are not present in boiled milk" (soft-curd).

Anthony (5) reported that the regurgitation subjects employed in his study noted a lack of distress from drinking soft-curd milk. Jeans (65) believed that the soft-curd character produced in milk by proper homogenization might be expected to lead to greater speed of digestion of milk and, thereby, possibly contribute to an earlier feeling of hunger and also,

possibly, to a relief from a feeling of overfullness that some adults seem to have as a result of drinking milk.

Spur and Wolman (99) successfully raised more than 200 normal infants on homogenized milk without the children exhibiting symptoms or signs of digestive disturbances attributable to imperfect utilization of the milk within the gastrointestinal tract. Sonic, low-pressure and high-pressure homogenized milks were used in their studies.

The role of homogenized milk during febrile illnesses. Milk usually is included in the dietary of patients during sickness. Some illness involving fever may alter the digestibility of milk since the acid of gastric juice decreases temporarily during fever (15). Wolman (134) discussed this possibility as follows:

"The intragastric milk clotting then becomes altered; the coagulation becomes affected by the pepsin enzyme which, when acting alone, tends to produce large calcium-rich curds. Facilitating this trend toward larger curds is the element of fever itself. As the environmental temperature rises above normal body temperature caseinous clots exhibit a marked tendency to shrink by syneresis and grow tough and rubbery. Thus can be explained the nausea and vomiting occasionally seen following the taking of pasteurized milk during febrile illnesses. This tendency, of course, is less marked with homogenized and other more soft-curd milks, which give rise to smaller, softer, and presumably more readily digested curds."

Calcium, phosphorus and nitrogen retention. Hess, according to Anthony (5), has indicated that reduction of the curd tension is a factor in inducing calcium retention, since it increased the availability of the vitamin D and the minerals in the milk itself by reducing the periphery and toughness of the curds. However, Jeans *et al.* (68), in feeding infants milks which permitted production of fine curds in the infant's stomach (acidified, pepsin-rennin and reconstituted evaporated but not homogenized milk), found: (a) normal growth rate, both in length and weight; (b) normal development and calcification of bone; and (c) a steady increase of retention of the elements studied, all of which constituted evidence that all of these milk mixtures were good for infants. However, in these studies a soft-curd character was obtained by means other than homogenization.

Homogenized certified milk. Elias (44) concluded that soft-curd milk had no decided advantages over other certified milk in digestibility and that it had no unusual tendency to make infants gain weight. On the other hand, Corbin (30) reported favorable results on the use of natural soft-curd certified milk in infant feeding, citing 11 cases of infants ranging from 2 months to 3.5 years.

Wolman (128, 129) believed that physicians would have complete confidence in the soft-curd nature of special homogenized certified milk which could be prescribed for dietary purposes, since certified milk would guarantee to the consumer that the product was all that it was represented to be, not adulterated with reconstituted milk or deficient in butterfat.

Effect on appetite when homogenized milk is taken between meals. A thesis presented before the American Pediatric Society in 1940 indicated that milk required 3 to 3.5 hours for complete gastric digestion and that routine feeding of milk midway between meals was not advisable (134). In extensive studies, Wolman (134) observed that before-meal feedings of nonhomogenized or homogenized milk failed to elicit in any child any undesirable symptoms of anorexia, gastrointestinal distress, or decreased consumption of food. For nearly 5 months, 59 convalescent children (3 to 14 years of age) were given a 7-ounce glass of milk twice daily, 1 hour before meals, homogenized milk (mean curd tension, 10.7 g.) and pasteurized milk (mean curd tension, 33.6 g.) being served in alternate months. Concerning the reported unfavorable experiences sometimes encountered with milk before meals, he comments, in part, as follows:

"One hypothesis for the recounted unfavorable experiences with milk before meals suggests itself. In extremely cold weather and under special circumstances, market milk sometimes develops a high content of casein and fat and grows markedly hard-curd. Such milk—of curd tension often over 50 gm.—may give rise to tough rubbery clots inside the stomach. Occasionally a child may suffer from a temporary reflex loss of appetite as a result."

A later report by Wolman (135) on the physiology of milk digestion during childhood showed that following the drinking of 8 ounces of milk the gastric-emptying time ranged from 50 to 170 minutes, the mean of 122 experiments being almost exactly 120 minutes. No appetite-impairing effect from milk occurred.

The effect of drinking milk between meals on the quantity of food taken spontaneously at mealtime. Wolman (134) subjected 18 children to a quantitative 3-week diet-intake study in which extra milk, pasteurized and homogenized, was given in 7-ounce quantities 1 hour before each meal for 2 of the 3 weeks, and the amount of food taken spontaneously during meal-times was measured carefully. He found that the feeding of either type of milk before meals had no effect on the amount of food consumed at meal-times. Thus, the data indicated that extra servings of milk might improve the child's nutritional status or food intake, and homogenized milk was neither superior nor inferior in this respect.

Increased consumption. Irwin (63) reported that after the introduction of homogenized milk at the Mont Alto Sanitarium in Pennsylvania, the consumption of milk increased from 1.5 to 2 quarts of milk per capita per day. Likewise, Hollingsworth (59) reported an increase in milk consumption. He stated, "There is no doubt but that homogenization of milk has increased the per capita consumption of milk and milk products. A pleasing glass of milk of uniform quality and richness has a tendency to enhance, everyone will admit, the consumption of that product. Homogenization has brought about increased consumption of milk products."

Wolman (134) believed that the improved nutritional quality as a result of the homogenization process would be reflected in an increased volume of consumption.

VI. HOMOGENIZED MILK IN INFANT FEEDING

Introduction of and emphasis on soft curd. Many of the observations and researches, especially the earlier ones, on the use of cow's milk in infant feeding centered around soft-curd milk. Although some of the earlier studies had little to do with homogenized milk, they are included herein in order to present a more complete picture of the role of homogenized milk in infant feeding. An exception is the early observation of Variot (117) who, noting the soft, diffuent, creamy curd formed from homogenized milk, successfully administered homogenized milk to a hundred nursing babies of various ages, some having more or less gastro-intestinal disorders. However, it was in the feeding of newborn and debilitated infants afflicted with gastro-intestinal disturbances that homogenized milk was used most successfully. He observed, "Generally at the end of a few days, vomiting ceased, the stools acquired normal consistency and color, the growth in weight followed its normal course." Mention should be made, however, that homogenized milk at that time was sterilized and, except for concentration, likely was more similar to evaporated milk than to present day homogenized milk.

Apparently a quarter of a century was to pass before there was any renewed interest in the use of homogenized milk in infant feeding. Meanwhile, many studies were made on soft-curd milk.

Brennemann (17) demonstrated experimentally, using humans, that boiled, soft-curd cow's milk did not form large, hard coagula in the stomach as did the raw milk. As a result of extensive studies on the coagulation of cow's milk in many modifications in the human stomach, he (18) emphasized that "*cow's milk is not a liquid food, but a solid food—so solid, in fact, that in babies the curds found in the stomach often pass through the whole intestinal tract and appear in the stools as large, hard, beanlike curds.*" He noted in a series of human regurgitation studies that raw milk was a very solid food and that boiled milk was a semi-liquid food. The solidity of the curd could be modified by many methods. Dried and condensed milks, as a rule, formed a minimum curd. Later, he (19) summarized: "*It is known that cow's milk is a peculiarly solid food; that it coagulates in large firm masses in the stomach; that these coagula become larger for about two hours by agglutination and continuously and increasingly harder by contraction and that all digestion is at the periphery only. This is as true of boiled as of raw milk, of diluted as of whole milk, except that in each former case all of the changes are less marked. Nowhere else would one venture the opinion that the size and consistency of the ingested*

bolus, and especially of such a bolus, would not be an important factor in digestion."

Buckley (20) believed that the inability of infants to digest and assimilate raw untreated milk from some cows and to assimilate similar milk from other cows was due to the curd character of the milk.

Dennett (31) concluded from clinical studies that boiled milk aided in overcoming digestive disturbances in infant feeding and that it did not cause digestive disturbances in normal infants.

Hill (54) early pointed out that the curd tension of milk might serve as a guide in the selection of milk for infant feeding and (55) that the physical curd character, as determined by the curd test, was an index to the comparative digestibility of the milk by delicate infants. Later, in discussing the nutritional advantages of natural soft-curd milk, he (56) stated, "If it is possible by supplying soft-curd milk to obviate the necessity of using a formula and to allow the use of the milk as it comes from the bottle, a great service will have been rendered to mankind. Soft-curd milk is not confined to infant nutrition alone. In case of adult indigestion and in gastric ulcers it has been used with remarkable results. It can thus become a boon to invalids and mature persons in general as well as to infants." Summarizing a decade and a half of soft-curd studies, he (57) reported that feeding tests with soft-curd milk conducted in different sections of the United States have been favorable to the use of soft-curd milk. He stated that when infants are fed a natural unmodified milk they do not acquire a dislike for milk as they grow older, as often is the case when they are fed a sweetened modified milk.

Barnes (9), with limited observations of babies fed on soft-curd milk, stated that the results seemed to show that this type of milk feeding was superior to hard-curd milk and that quite possibly many difficult feeding problems could be solved by using it.

Scales (91) stated that soft-curd milk formed a soft, flaky mass in the stomach in contrast to the coherent, rubbery mass of hard-curd milk which caused regurgitation and indigestion to the infant.

Elias (44) concluded from his observations on feeding 82 babies for periods from 10 to 90 days on soft-curd milk (natural soft-curd, boiled certified and evaporated) that soft-curd milk had no decided advantages over other certified milks in digestibility and that it had no unusual tendency to make infants gain weight. Neither did it have any special value in preventing or treating vomiting and diarrhea.

Doan (33), summarizing the advantages of natural soft-curd milk, believed that since babies having difficulty with ordinary milk, showing no gain in weight and troubles with regurgitation, almost without exception ceased vomiting and began to gain as soon as soft-curd milk was used; and since patients in hospitals and sanitoriums were usually subnormal and

many were afflicted with poor digestion, that soft-curd milk would be demanded in the future for these groups of consumers.

Based upon studies on a group of 60 infants ranging from birth to 6 months of age fed on soft-curd milk for a period of time ranging from 2 to 8 weeks (44 were fed over a 7-week period), Morris and Richardson (85) observed that normal milk of low curd-tension was low in energy value and when infants were fed to satiety they needed more normal soft-curd milk than ordinary milk. Thus, they concluded: "Considering (a) the variability in the production of soft-curd milk and the increased observation necessary in its production; (b) the lack of superior results when used in comparison with other accepted infant formulas, soft-curd milk does not warrant special production and certification for use in infant feeding."

The Committee of the American Dairy Science Association (1) on methods of determining the curd tension of milk advised that the curd tension value of milk should not be considered an absolute index of its digestibility or of its suitability for infant feeding purposes. They believed, however, that the values did correlate in a general way with those properties and, thus, the determination appeared to be the best simple method for the purpose available.

Marriott and Jeans (81) stated: "Soft-curd milks are advantageous in that they produce a softer, finer curd in the stomach, and therefore leave the stomach more quickly and are more quickly digested. This advantage, however, is largely for the person past infancy, since all milk for infant feeding should be boiled, and boiling is effective in reducing curd-tension sufficiently for the milk to have soft-curd properties."

Introduction and use of homogenized milk for infants. Washburn and Jones (121), noting that curds from homogenized milk were so much more flocculent and friable than those from nonhomogenized milk, believed that nutritional benefits might be expected from such treatment.

Eichholz (43) recommended homogenized milk for infants and for forced feedings. However, the milk used by him, in addition to being homogenized, was sterilized, which would reduce further the curd tension.

Clay (28) reported that homogenized milk gave good clinical results in cases of difficult feeding. He believed that if a milk of low specific gravity and low curd tension were homogenized, with the resultant subdivision of the fat globules and the reduction of the curd tension to 10 g. or lower, a raw milk very suitable for infant feeding would result. (Danger of rancidity resulting from homogenization of raw milk was not widely known at that time.)

Berry (11), in two feeding trials with rats, noted in both trials that the rats fed normal hard-curd milk which was homogenized to produce a soft curd made the largest average gains.

Wolman (127) stated, "These new products (soft curd and low tension

milks) break down the last obstructing fortress on the road to the perfect digestion of milk and represent an important contribution to the healthful feeding of infants, children and sick adults. Improved nutritional quality will be reflected undoubtedly in an increased volume of consumption." Later he (130, 131) concluded that pasteurized homogenized milks used in their studies were found to be "as good or better as a food for infants than pasteurized milk boiled for five minutes in the home. Such mechanically processed milks may be safely fed to infants and young children; in fact the danger of accidental household contamination is less than when unprocessed milk is employed." However, he (131) felt that over the country as a whole the status of homogenized milk was not yet at such a uniformly high peak that indiscriminate feeding of normal babies without boiling of the milk could be considered safe. Nevertheless, the fact cannot be ignored that homogenized milk must be a pasteurized product (22, 42, 51) and that the addition of raw milk invariably causes a rancid flavor (42, 49, 79), thus adversely affecting its palatability and reducing its consumption.

Later, Wolman *et al.* (136) compared homogenized milk (sonic, low pressure, and high pressure) with boiled, pasteurized milk as a base for infant formulas in feeding trials with over 200 infants for 2 to 12 months. They observed a low incidence of constipation, diarrhea, vomiting and kindred gastro-intestinal upsets in all groups; in digestibility and safety, the homogenized milks proved as satisfactory as the control boiled milk.

Wilson (126) concluded that soft-curd milk seemed to have special value for infant feeding and for some older children and adults, but he questioned if one method of producing soft-curd milk was superior in all respects to any other method.

Kugelmass (76) noted that the tolerance of infants for milk has been enhanced by homogenization, which softened the curd by increasing the amount of casein adsorbed on the surface of the fat particles.

Jeans (66) believed that from the point of view of a pediatrician, homogenized milk had two advantages: the vitamin D of fortified homogenized milk went to those who drank the milk, and it was more rapidly digested because of the effect on the casein curd. However, he believed that most pediatricians would not consider homogenization important in infant feeding because milk formulas should always be boiled for bacterial safety, but, after the formula period, homogenization is useful in making milk more readily digestible.

Homogenized milk in the preparation of infant formula. The use of homogenized milk in the preparation of infant formulas seems to have found favor both with the mothers and with the physicians. Clay (28) advised that homogenization made it possible to use a soft-curd milk without having to boil the milk.

Wolman (127) stated that "homogenized milk makes an excellent foundation for infant formulas. . . . The curds obtained are always much finer than the curds of formula derived from identical specimens of milk pasteurized but not homogenized." Later (131) he noted that the making of formulas with homogenized milks was markedly simplified, stating: "Elimination of the steps of boiling, filtering and cooling saved the nurses and mothers an appreciable number of minutes each day, and reduced the opportunities for accidental household contamination of the contents of the formula bottles." Furthermore, making a formula with the cold milk (homogenized) was simpler and better than heating and allowing the prepared formula to cool over an extended period, thus permitting bacterial growth (136). By use of holding pasteurization temperatures from 150 to 160° F. for 30 minutes, the bacterial counts of homogenized milks could be kept at low levels, usually below 2,000 per ml., resulting in high sanitary quality and eliminating introduction of an objectionable heated flavor (136).

Blatt (14), following an 18-month study, concluded that commercial pasteurized milk with a curd tension reduced to below 20 g. by proteolytic enzyme action was desirable for infant feeding, since it was well tolerated and digested and, in addition, was easily, economically and safely prepared. He believed also that in its preparation the bacteriostatic value of handling a food product at a low temperature was utilized in contrast to the practice of making the milk soft curd by boiling and allowing it to cool slowly.

Glynn (48), commenting on the observations of Conquest *et al.* (29), who found that low curd tension milk yielded a soft curd which passed through the stomachs of calves more rapidly than normal curd tension milk, stated that successful feeding for each species must employ a milk of curd tension physiologically adequate for the gastric digestive apparatus of that species. Thus, he believed milk for successful infant feeding should have a soft-curd character.

Espe and Dye (45) pointed out that normal milk of high curd tension usually meant a more concentrated milk than one of low curd tension and, therefore, one of greater food value. Thus, a hard-curd milk rendered soft by homogenization would seem to have a greater caloric value than a natural soft-curd milk.

DISCUSSION

The general acceptance of homogenized milk would indicate that the nutritive value of the milk is not impaired. On the other hand, the possible enhancement of the nutritive value of milk by homogenization would seem to depend upon the importance in nutrition of the roles of such factors as palatability, homogeneity, satiability, digestibility, and retention and utilization of its constituents.

Probably the chief value of homogenization of milk from the nutritive standpoint is the fact that the process renders the milk homogeneous throughout. There can be no pouring off of the cream layer, leaving the partially defatted milk for those less fortunate. However, should there be no need for butterfat calories, this property becomes a distinct disadvantage. Properly homogenized milk always will carry its full caloric value because the fat cannot be removed by pouring. Associated with the milk fat are the vitamins A and D. Those who get the fat get these vitamins. Homogenized milk is better suited for vitamin D fortification than is nonhomogenized milk, for the added vitamin D associated with the fat will remain uniformly distributed in such milk. Children drinking vitamin D-fortified homogenized milk will be assured of their portion of vitamin D, an important factor in nutrition.

A second important property of homogenized milk is its palatability. Homogenization fixes the fresh flavor of milk so that the fat of milk does not oxidize readily, yielding the old, stale, cardboard, oxidized flavor which is particularly objectionable to children. Not only does the process fix the flavor of milk at time of homogenization, but it stabilizes the fat of adequately processed and properly stored homogenized milk over a long period of time. In every-other-day delivery, this is especially important. When the milk is several days old, it is as palatable as when first produced. Also, there are no thick cream plugs, flecks or butter granules floating on a glass of homogenized milk. To children these seemingly foreign particles are particularly nauseating and objectionable. Casual reports and general observation, not based on scientific evidence, indicate that children take to homogenized milk because it is sweet, smooth and uniform in consistency. Generally, there is less of a milk-drinking problem when children have access to homogenized milk.

Homogeneity and palatability would seem to be major advantages of homogenized milk effecting greater consumption of milk and, therefore, improving human diets. However, these qualities are often overlooked or overshadowed by the claims of "greater digestibility", "more digestible", and so on, as a result of the softening of the curd by proper homogenization. Claims that homogenization makes the protein and fat of milk more completely digestible seem to lack the support of adequate experimental evidence obtained in controlled feeding trials. Nevertheless, data indicate a slightly faster stomach-emptying time when homogenized milk was ingested than when nonhomogenized milk was taken into the body. The desirability of rapid stomach-emptying time is sometimes questioned, some believing that homogenized milk does not "stick to the ribs" and hunger soon manifests itself. However, rapid stomach-emptying time would seem to be an advantage, especially in infant feeding. Associated with rapid stomach-emptying time seems to be a lack of distress after ingesting homogenized

milk. That homogenized milk plays a real role in infant feeding is supported by observations made by authoritative pediatricians.

SUMMARY

An attempt has been made to make this review as complete and unbiased as possible. To this end opinions, observations and data bearing on the subject have been gleaned from the literature. That many data were conflicting is a foregone conclusion. Nevertheless, the majority of the data reported herein would seem to support the following conclusions concerning homogenized milk:

1. The homogeneity of properly processed homogenized milk assures equal distribution of the fat, vitamin A, and added vitamin D to those who consume the milk.

2. Homogenization retards or inhibits the development of the copper-induced oxidized flavor over an extended period of time. Thus, the milk properly stored and refrigerated retains the fresh flavor which contributes much to its palatability.

3. The smooth, uniform consistency of properly homogenized milk, as shown by the absence of cream flecks, non-miscible cream or butter granules, further contributes to its palatability.

4. Proper homogenization changes normal hard-curd milk to a soft-curd milk, which appears to be digested slightly more easily but neither more quickly nor more completely than hard-curd milk despite a faster stomach-emptying time.

5. Reduction in the size of the fat globules by homogenization does not increase the digestibility of the fat, since the digestibility of the fat of fresh whole milk is so nearly complete that any marked increase in digestibility is not possible.

6. Homogenized milk has been used successfully in the preparation of infant formula and in infant feeding.

7. The ingestion of homogenized milk seems to be associated with a lack of distress and a sensation of overfullness during digestion. Hence, homogenized milk would seem to be especially suited for hospital diets.

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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-THIRD ANNUAL MEETING

PRODUCTION SECTION

- P1 The Relative Merits of a Cow's Own Record and Her Progeny Test for Predicting the Butterfat Production of Her Future Daughters.** W. J. TYLER AND GEORGE HYATT, JR., West Virginia University.

Wide differences of opinion now exist concerning the relative importance of a cow's production records and her progeny test in estimating a cow's breeding value or transmitting ability. The objective of this study in dairy cattle breeding was to compare the relative value of one unselected record of a cow and her daughter's records in estimating butterfat production of future daughters.

Data consisting of first records on 1,249 Ayrshire cows and their daughters were analyzed to determine the relationship between one unselected record of a cow and the production of her daughter. Also, correlation coefficients between the production records of one, two or three daughters and the records of additional daughters were calculated. The results indicate that one unselected record of a cow may be as important in predicting the production of her daughters as the average of the records of two or three daughters are in estimating the butterfat production of additional daughters. The intra-herd correlation coefficients varied from 0.10 to 0.26, while the gross correlations were from 0.18 to 0.38. A combination of the record of the cow and a record for one or two daughters was better than either one alone in predicting the production of future daughters.

- P2 Preliminary Results from the Crossing of Two Inbred Lines of Holsteins on Growth and Milk Production.** N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, University of California.

The University of California Holstein herd was inbred for 11 years through sire-daughter matings. Females with a relatively high coefficient of inbreeding (Wright's— F) were smaller at birth, had a higher mortality rate, and developed into smaller mature animals than the outcross generation. Butterfat production decreased with each successive generation of sire-daughter mating until females with an F of 37.5 and above produced 199 lb. less than the first-generation daughters or 206 lb. less than the foundation females.

An unrelated sire ($F = 23$) mated to the first sire's inbred daughters (average $F = 29.8$) produced offspring averaging 13.4 lb. heavier at birth than their dams and 6.2 lb. heavier than the foundation cows. Such off-

spring also made more rapid growth and attained a greater ultimate size. Butterfat production of these outcross cows was 203 lb. more than for their dams and 52 lb. greater than for the foundation cows. This sire is being mated to his daughters in an attempt to determine the reason for the increase in growth and butterfat production. Thus far, birth weights and body measurements indicate that heterosis is involved.

P3 Genetic Variation in the Levels of Blood Plasma Carotene and Vitamin A in Dairy Cattle. R. E. MATHER, University of Wisconsin.

Blood samples were collected from calves, heifers, and cows from one Holstein and two Guernsey herds at intervals averaging 5.5 weeks from June until August of the following year. The plasma was analyzed for total carotenoids and vitamin A. The relationships of carotene and vitamin A with season, age of the animals, level of milk production and length of period in milk were studied. Appropriate corrections then were made and the heritability within season was determined by correlations between related animals.

Combining the correlations for the different age and breed groups through the "z" conversion and including two negative values gave an average correlation between carotene levels of daughter and dam of 0.1324, which was significant for 230 degrees of freedom. For vitamin A the average correlation was 0.1555, which also was significant. Doubling these values gave "heritabilities" of 0.26 for carotene and 0.31 for vitamin A.

P4 Measurement of the Rate of Endocrine Gland Secretion as a Tool in the Genetic Selection of Dairy Cattle.¹ C. W. TURNER, Missouri Agricultural Experiment Station.

Genetic improvement in the "capacity for milk and fat production" of dairy cattle has been retarded seriously due to the lack of measures of the several factors which combine to make possible high production. Since it now has been demonstrated that the growth of the udder, the initiation and maintenance of milk secretion, and the "let down" process are controlled by the hormones secreted by the various endocrine glands, it is obvious that the rate of secretion of the several hormones is the key to the problem.

The problem can be broken down into the following component parts:

<i>Hormones</i>	<i>Controls</i>
1. Estrogen	Act upon anterior pituitary Udder size, amount of se-
2. Progesterone	to secrete mammogen. creting tissue.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1110.

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|------------------------------|-----------------------------------------------------|--------------------------------|
| 3. Lactogenic hormone | Acts upon udder cells to secrete milk. | Persistency of milk secretion. |
| 4. Other pituitary hormones | Stimulate the production of the precursors of milk. | Secretion of milk. |
| 5. Thyroxine | Acts upon blood supply. | Increases secretion of milk. |
| 6. Parathyroid hormone | Regulates the Ca and P of the blood. | |
| 7. Adrenal cortical hormones | Mode of action not clear. | Essential for lactation. |
| 8. Pituitrin | Acts upon the "let down" of milk at milking time. | |

When all of the hormones involved in these processes are being secreted in optimum amounts, one might expect high milk production. The secretion of any one hormone in limited amounts will limit the capacity of the animal. Methods of detection of the limiting hormones will be explained.

P5 Thyroid Secretion Rate and Its Relation to Various Physiological Processes.¹ VICTOR HURST AND C. W. TURNER, University of Missouri.

The knowledge of the thyroid secretion rate in an animal aids in understanding the relationship of the thyroid gland to various physiological functions such as growth, reproduction and lactation. In the growing mouse, the administration of thiouracil retards growth, but animals maintained normal growth when receiving thiouracil plus thyroxine injected in amounts approximating the normal thyroid secretion rate. Injecting thyroxine in larger amounts stimulated growth as long as physiological dosages were administered.

The thyroid secretion rate per 100 g. body weight declined during the growth period, and there were indications that the reduction of thyroxine dosage during a rapid period of growth coinciding with a declining thyroid secretion rate stimulated growth to a greater extent than did the administration of a constant dosage of thyroxine over the same period. Thyroid secretion rate varied among mature mice according to strain, and within a given strain there were sex differences in thyroid secretion rate. Castration decreased and the feeding of dianisylhexene (dimethyl ether of diethylstilbestrol) increased the thyroid secretion rate.

Evidence is presented to show that thyroxine does not pass through the mammary gland of the mouse into the milk. Lowering the environmental temperature below the zone of thermoneutrality in the mouse increased the thyroid secretion rate in both males and females. Approximately 7 per cent of the thyroxine in thyroprotein was utilized by mice when it was administered orally, as compared to subcutaneous injection.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1108.

These studies emphasize the importance of using the normal thyroid secretion rate of animals as a reference base in studying the effects of hypo- and hyperthyroidism upon the various physiological processes of the animal body.

P6 The Effect of Low Levels of Thyroprotein Feeding upon Milk and Milk Fat Production, Body Weight, Body Temperature, Heart Rate and Respiration Rate of Dairy Cows. R. G. SWANSON AND C. B. KNOTT, The Pennsylvania State College.

Two trials have been conducted to determine the effects of feeding daily 0.625, 1.25 and 5.0 g. of thyroprotein for 1 year to dairy cows. In the first trial, 12 Holstein cows were divided into four comparable groups. These groups were assigned at random to the several levels of thyroprotein feeding, as well as to a control group. The T.D.N. intake of the cows was 125 per cent of the Morrison Standards for good cows under usual conditions. On the basis of 4 per cent F.C.M., M.E., there was no significant difference in milk production between the thyroprotein-fed groups and the control group. No differences were found between the groups in body temperature, heart rate, respiration rate, gain in body weight or breeding efficiency.

The second trial was conducted with 32 cows divided into four comparable groups. In this trial Ayrshire, Brown Swiss and Guernsey cows were used. Their T.D.N. intake was 110 per cent of Morrison's Standards for good cows under usual conditions. Thyroprotein was fed at the same levels as in the first trial for 1 year, including the dry period. All groups gained in body weight and had similar body temperatures, heart rates, respiration rates and breeding efficiency. At this level of T.D.N. intake (110 per cent), thyroprotein appeared to stimulate milk production.

P7 Effects of Feeding Thyroprotein to Milking Cows in Summer. K. E. GARDNER AND T. W. MILLEN, University of Illinois.

In an experiment run during the hot summer weather of 1947, 12 cows receiving thyroprotein¹ at the rate of 1.33, 1.5 and 2.0 g. daily per 100 lb. body weight showed fat yield increases of 32, 48 and 50 per cent, respectively. During July and August, the cows fed thyroprotein lost weight rapidly, although receiving 140 per cent of Morrison's minimum T.D.N. requirement. When the weather cooled, large weight gains occurred on intakes above 130 per cent of requirements.

Body temperatures of Holsteins receiving thyroprotein averaged 105° F. when the environmental temperature reached 96° F. and averaged 1.5° F. higher for the trial. Cows on the highest rate of thyroprotein exceeded respiratory rates of 100 per minute on the hottest day and averaged

¹ The thyroprotein used was "Protamone", furnished by the Cerophyl Laboratories, Inc., Kansas City, Missouri.

78 compared to 49 for controls. The pulse rates averaged 95 compared to 49 for controls. There were no outstanding differences in blood pressure as measured using the broad cuff on the tail (Harshbarger, University of Illinois, unpublished). Values for hemoglobin, serum calcium and inorganic serum phosphorus showed no trends attributable to the thyroprotein feeding. All cows, including controls, showed a marked lowering of serum calcium and magnesium in midsummer and a rise in September.

P8 Effects of Feeding Thyroprotein during Successive Lactation. J. W. THOMAS AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

Thyroprotein has been fed to 12 dairy cows for successive lactations, beginning at 50 days postpartum and continuing until 90 days before the next parturition. All the cows were normal at parturition time and all the calves appeared normal.

Milk production during the first 50 days of successive lactations was compared. During this segment of the second and third lactations, the cows fed normally produced 132 per cent as much milk as they produced during their first lactation. Cows that had been fed thyroprotein for one or two lactations produced only an average of 117 per cent during the first 50 days in their second and third lactations as compared to the first lactation.

The average butterfat test during the first 50 days of the second and third lactations for 13 normally fed cows was 106 per cent of the value for the first lactation. A corresponding value of only 89 per cent was found for eight cows that had been fed thyroprotein during their first and second lactations. In the second and third lactations following thyroprotein administration, an increase in milk production and butterfat percentage always was observed. However, the total production during the second and third lactations was not much greater than that observed during the first lactation.

Production data and plasma protein-bound (thyroxine) iodine values indicate that the thyroid gland may be functioning at a subnormal rate for at least 140 days after the cows have received thyroprotein for one or two lactations.

P9 Factors Controlling the Extent of Duct Growth in Mammary Glands.
I. The Influence of an Estrogen in a Hereford Heifer. RALPH P. REECE, New Jersey Agricultural Experiment Station.

Of two sexually immature Hereford heifers, one was injected subcutaneously once weekly for 16 weeks with 5 mg. of estradiol dipropionate.

Six days after the initial injection the experimental heifer was in estrus; however, a rectal palpation of the ovaries indicated that they were immature. Estrus also was observed in the control heifer during the experiment. At the time of slaughter, the udder, teats, and pituitary gland of the injected heifer were larger than those of the control heifer. Histological preparations showed a greater hyperplasia and hypertrophy of the glandular tissue near the gland cistern in the injected heifer than in the control heifer. The distance that the duct system extended from the base of the teats was essentially the same in the two heifers. Ovulation had occurred in the control heifer but not in the injected heifer.

P10 The Value of Oxytocin for Reducing Fluctuation in Milk and Fat Yield during Experimental Periods. H. P. ADAMS AND N. N. ALLEN, University of Wisconsin.

The daily variations in milk and fat production and in fat percentage confuse the measurement of small differences in these values resulting from experimental treatment. These variations are due in part to incomplete removal of the milk from the mammary gland.

This experiment, using eight cows, was conducted to determine whether the use of oxytocin to insure complete milking will reduce materially the daily variations. A reversal plan was followed with 10-day experimental periods. Under normal feeding, oxytocin administered prior to milking did not reduce significantly the variability in milk or fat yield or fat percentage, as compared to a good milking routine without oxytocin. Comparing high and low fat intakes, the effect of the high fat intake on fat production and test was shown as clearly without as with oxytocin. While the oxytocin did not reduce variability, it did cause a highly significant increase in milk and fat yield, with no significant effect on fat percentage.

In order to secure information as to why the extra milk, presumably residual milk of high fat content, did not increase the test, cows were milked in three approximately equal portions, which were tested separately. Under normal milking, the first portion had the lowest and the last portion the highest fat content.

During a second period, oxytocin was injected following the regular milking to secure any residual milk remaining. After the first oxytocin milking, it was found that the amount of milk in the third portion before injection was reduced. The residual milk secured following injection was higher in fat than the third portion, but the first portion of the succeeding milking was abnormally low in fat content, offsetting the effect of the high fat, residual milk. This is interpreted as indicating that the residual milk under ordinary milking practices is secured in the normal milk of the following milking.

P11 The Role of Certain Hormones in Spermatogenesis.¹ J. D. SAMPATH KUMARAN, University of Missouri.

The investigations reported deal with the effects of various hormones on growth and development of the testes and spermatogenesis. The experimental animals were White Plymouth Rock cockerels. In these birds, the appearance of the first primary spermatocyte was at 42 days, the secondary spermatocytes on the seventieth day, and spermatids on the eighty-fourth day after hatching. During the winter this process was delayed about a week. Through the use of the polarizing microscope, birefringence was observed in the region of the cells of Leydig. Since the steroid hormones give this reaction, it has been interpreted as indicating that the male hormone is secreted by these cells.

Hypothyroidism, induced by feeding 0.3 per cent thiouracil in the ration, started on the 12th week and continued for 30 days, inducing regression of testis size and spermatogenesis. When testosterone at the rate of 20 mg. per kg. feed was fed with 0.3 per cent thiouracil, testis size was brought back to normal. Thus thiouracil does not possess toxic effects at this level of administration which cannot be corrected by the thyroid hormone; this hormone in amounts above normal actually stimulates growth of the testes and spermatogenesis. The feeding of the dimethyl ether of diethylstilbestrol at the rate of 4 mg. per 100 lb. feed to the same type of birds caused regression of the testes to one-tenth the normal size. The feeding of 10 per cent dried cow manure did not correct this condition.

P12 The Relationship between Type Ratings of Ayrshire Females as Young Heifers and as Cows. GEORGE HYATT, JR., AND W. J. TYLER, West Virginia University.

Since type is heritable and important to breeders of dairy cattle, it is very desirable that dairymen be able to determine which are the poorest type animals in their herds at as early a date as possible. A cooperative project was inaugurated in 1942 by the West Virginia Agricultural Experiment Station and the Ayrshire Breeders' Association to determine the repeatability of heifer classifications at 6-month intervals and the correlation between the several classifications of a heifer and her classification after freshening.

One hundred and two Ayrshire heifers, each of which has been classified at least once following freshening, have been rated for type each 6 months during the period from October, 1942, through April, 1948. The inspectors classified each heifer into one of five groups (Excellent, average score 90-100; Very Good, 85-90; Good Plus, 80-85; Good, 75-80; Fair,

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1107.

70-75), similar to the classification program of adult animals. When these 102 animals were placed in four groups (Group I, below 80.5; group II, 80.5-82.5; Group III, 82.6-85.0; and Group IV, above 85.1) according to the averages of their several ratings before calving and when these averages were compared with the group average after freshening, the following results were obtained: Fourteen animals in Group I averaged 78.6 before freshening and 80.1 after freshening; 34 in Group II, 82.0 before freshening and 81.5 after freshening; 31 in Group III, 84.4 before freshening and 83.0 following freshening; and 23 in Group IV, 87.1 before freshening and 84.5 after freshening. The correlation between the several ratings of the heifers before calving with their ratings after calving was approximately 0.35, which is only slightly lower than the correlation between the consecutive ratings of mature cows when rated by different judges.

P13 Effect on the Blood Vitamin A Activity Values for Dairy Animals of Certain Vitamins and Minerals. DWIGHT ESPE, D. W. BOLIN, AND F. M. BOLIN, North Dakota Agricultural College.

Thirty dairy cows were fed for 18 weeks a ration of prairie hay, corn silage, grain, a carotene concentrate (equivalent to 90 mg. of carotene daily), and certain vitamins or minerals. At the rates fed, cobalt, iodine, or *alpha*-tocopherol had no influence on the carotene and vitamin A values of the blood of these cows. The adding of a small amount of vegetable oil to the carotene concentrate did not alter the carotene and vitamin A values occurring in the blood.

Twelve heifers were fed prairie hay, grain and a carotene concentrate or dehydrated alfalfa. Forty-five mg. of carotene daily in the carotene concentrate or dehydrated alfalfa hay which was fed exerted a negligible effect on blood carotene and vitamin A values.

P14 Effect of Certain Soybean Products on the Concentrations of Carotene and Vitamin A in the Milk Fat and in the Blood Plasma of Dairy Cows. R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN, Iowa State College.

Two trials were conducted using paired Holstein-Friesian cows to determine the effect of raw soybeans and soybean oil in the first trial and the effect of raw soybeans and soybean oil meal in the second trial on the concentration of carotene and vitamin A in blood plasma and milk fat. Feeding raw soybeans in amounts of 9 lb. daily per lactating cow caused the blood plasma concentrations of carotene in these cows to be markedly lower than those concentrations of carotene in cows fed the control ration containing no soybean products. These differences, in both trials, tended to level off after about 6-8 weeks of feeding the beans.

Measurable differences occurred between the blood plasma carotene concentrations of cows fed soybean oil (1.7 lb. daily per cow) and the controls, the cows getting the soybean oil having the lower concentration. These differences were not so large as those caused by raw soybeans. Soybean oil meal caused no great change in carotene concentration in either the blood plasma or milk fat from the control ration. Raw soybeans and soybean oil caused differences in the concentrations of carotene in milk fat which were similar in direction to those found in the blood plasma when these feeds were compared with a control ration. The rations caused small variations, with no particular trends in the vitamin A concentrations of the blood plasma and in the milk fat.

P15 Further Studies on the Relationship between the Feeding of Soybeans and the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE, J. C. SHAW, AND B. C. HATZIOLOS, University of Maryland, AND L. A. MOORE AND J. F. SYKES, Bureau of Dairy Industry, U. S. Department of Agriculture.

Calves fed soybeans heated to 100° C. for 15 minutes exhibited a lower plasma vitamin A and a lower liver vitamin A than control calves which received an equivalent amount of raw soybeans. The feeding of 1 g. of iodinated protein per 100 lb. of body weight did not prevent the decrease in plasma vitamin A. Ayrshire and Holstein calves receiving 32 γ of carotene per lb. of body weight exhibited an increased spinal fluid pressure when soybeans constituted 30 per cent of the ration. These animals also exhibited testicular degeneration. Data will be presented on blood plasma and liver vitamin A levels of calves on a soybean ration in which the carotene was replaced by a vitamin A concentrate.

P16 The Influence of Tocopherols on the Fat Content of Milk. F. WHITING AND J. K. LOOSLI, Cornell University.

Experiments using 16 dairy cows representing the Holstein, Guernsey, and Brown Swiss breeds were carried out to study the influence of tocopherols (vitamin E) and cod-liver oil upon butterfat production. When tocopherols were fed at the rate of 1 g. per cow daily over a 4-week experimental period during winter feeding, the percentage fat in the milk was increased slightly. Cod-liver oil fed at the rate of 5 ounces per cow daily decreased the fat percentage approximately 11 per cent. Feeding tocopherols to cows fed cod-liver oil did not prevent the fall in butterfat percentage. Total milk production (lb. of 4% F.C.M.) was not significantly affected by feeding either tocopherol or cod-liver oil. Feeding the same amount of tocopherol to cows on pasture slightly increased the fat test of the milk but had no influence upon total milk production. Feeding toco-

pherols alone or in combination with cod-liver oil increased the tocopherol content of the butterfat produced, but had no apparent influence upon the vitamin A or carotene content of the fat. Feeding cod-liver oil increased the vitamin A content but decreased the tocopherol and carotene content of the butterfat.

P17 Covitamin Studies of the Milk Fats from Four Breeds of Dairy Cattle. V. N. KRUKOVSKY AND F. WHITING, Cornell University.

A study was made of the relationship between the tocopherol, vitamin A and carotenoid content of milk fat from four breeds of dairy cows. The analyses were made on individual fat samples obtained from 40 cows at the end of pasture feeding and again after 5 months of winter feeding.

Large variations were found in the content of these vitamins between individual cows of the same breed, between the different breeds, and between seasons. The following average values and standard deviations of tocopherols, carotenoids and vitamin A were found for each of the following breeds at the end of pasture season (all values $\gamma/100$ g. of fat): Holsteins, $2,253 \pm 822$, 504 ± 249 , and 546 ± 145 ; Brown Swiss, $2,860 \pm 656$, 785 ± 221 , and 703 ± 146 ; Guernseys, $3,164 \pm 462$, $1,583 \pm 237$, and 381 ± 120 ; and Jerseys, $3,036 \pm 498$, $1,236 \pm 358$, and 578 ± 123 , respectively. After 5 months of winter feeding, the corresponding values were as follows: Holsteins, $2,011 \pm 341$, 290 ± 109 , and 398 ± 86 ; Brown Swiss, $2,149 \pm 487$, 341 ± 165 , and 383 ± 47 ; Guernseys, $2,329 \pm 343$, 772 ± 169 , and 312 ± 109 ; and Jerseys, $1,905 \pm 361$, 341 ± 108 , and 301 ± 26 , respectively.

A highly significant correlation was found between the tocopherol and carotenoid content of the fat. However, no such relationship existed between tocopherol and vitamin A.

P18 The Effect of Lactation and Gestation on Heat Production and Cardiorespiratory Activities of Dairy Cattle and Rats.¹ SAMUEL BRODY, D. M. WORSTELL, A. C. RAGSDALE, AND H. H. KIBLER, University of Missouri.

Heavily lactating cattle and rats produce about twice as much heat under normal feeding conditions as those not lactating. Gestation increases heat production in cattle and rats about 40 per cent above the non-gestating level but only during the last third of the gestation period. The course of pulse rate, respiration rate and ventilation rate parallels the course of heat production. However, the tidal air tends to decline during the last third of gestation.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1111.

P19 A Biochemical and Histo-pathological Study of Ketosis in Dairy Cattle. J. C. SHAW, B. C. HATZIOLOS, AND V. P. SAARINEN, University of Maryland.

Blood samples were drawn from five cows which appeared to have ketosis and the animals were slaughtered for chemical and histopathological studies of various organs. Blood hemoglobin, red cell volume, phosphorus and chlorine were normal. Blood plasma cholesterol was low. The adrenals were low in ascorbic acid and normal in cholesterol. The total fat of the adrenals was high in all cases. Four of the five livers were high in fat and the kidneys were comparatively high in fat. The increased fat content of these organs was principally neutral fat, the cholesterol and phospholipid fractions being comparatively low. Liver glycogen was low in four of the five cases. The ascorbic acid of the adrenals was low, whereas the cholesterol was normal. Blood counts were made in three cases. The neutrophils were higher than normal and the lymphocytes and eosinophils low. The adrenals all exhibited marked fatty infiltration and partial degeneration, the heaviest degeneration being in the outer layer of the cortex, the arcata. The medulla of the adrenal was not affected. Marked fatty infiltration and partial degeneration also were observed in the pancreas, thyroids and the growing follicles of the ovary. One pituitary showed a rupture in the wall of the residual lumen resulting in a small sac filled with colloidal fluid. In another a depression was observed in the inferior part of the anterior lobe, apparently caused by outside pressure and resulting in a partial atrophy of the lobe. Data also will be given on paraffin sections. This constitutes a progress report only.

P20 A Study of Sampling at Various Stages of Milking in Determining the Bacterial Flora of the Udders of Dairy Cows. E. M. KESLER, C. B. KNOTT, AND J. J. REID. The Pennsylvania State College.

Strict foremilk was compared to samples obtained after various amounts of milk had been removed from the quarters. Nine hundred and forty samples were collected from 47 cows of varying age, production, stage of lactation and types of udder bacterial flora based on previous examinations. After careful washing of the udders with chlorine solution, five 15-ml. samples were drawn aseptically from each quarter as follows: strict foremilk, followed by two successive samples, midmilk, and strippings. Samples were incubated with brilliant green-sodium azide and then examined microscopically by means of a modification of the standard direct count method. All samples were streaked on Edwards medium, incubated, and examined for nature of growth if present. Further tests to identify the various streptococci found on the plates were run as necessary. These included isolation and cultivation in veal infusion broth, followed by

physiological and serological studies. No pronounced differences existed between strict foremilk and the second 15 ml. drawn from the teat. Not all of the long chain streptococci would have been noted had a sample of midmilk or strippings been used as the basis of the tests. Excessive numbers of leucocytes appeared more often in the last-drawn samples.

P21 A Permanent and Convenient Rumen Fistula for Dairy Cows. G. E. STODDARD AND N. N. ALLEN, University of Wisconsin.

Permanent rumen fistulas provide a very satisfactory means of studying digestion in the rumen, but there has been a need for a closure which will prevent leakage but which may be removed readily for sampling or observation. A cannula has been designed for this purpose. It is constructed entirely of transparent leucite, which is easily tooled and fabricated and at the same time resists corrosion and is non-irritating to the contingent tissue. A detachable base flange is threaded to the cannula to facilitate insertion, and a threaded collar is provided for adjusting the outer flange. A screw cap covers the 2-inch opening and is easily removed for observation or sampling. Special tools have been designed for insertion of the cannula at the time of fistulation.

Four cows have been fistulated and equipped with these cannulae. Leakage around the cannula has been slight. Three of the cows were fistulated during early pregnancy. One has freshened normally, and the other two are nearing termination of normal pregnancy. Cannulae of similar design have been used for one goat and a number of sheep.

P21-A Studies Bearing on the Bloat Problem. H. H. COLE, AND MAX KLEIBER, University of California.

In four 4-hour test periods, there was an average consumption of 47.6 lb. of sudan tops fed *ad libitum* in the barn with an average ruminal gas production of 4.7 cubic feet for the period. In four tests with alfalfa tops in the prebloom stage fed *ad libitum*, there was an average consumption of 15.5 lb. and an average of 4.3 cubic feet of ruminal gas produced. The rate of gas formation following the introduction of glucose into the rumen through a cannula has been compared with that of starch. When the cow was fed 9 lb. of alfalfa hay and 6 lb. of barley 20 hours before the experimental period, glucose caused a more rapid increase in gas formation than did starch, although the total gas produced within 4.5 hours after the introduction of either of the two substances was approximately the same, 3.4 and 3.1 cubic feet, respectively.

From pasture consumption studies, evidence was obtained indicating that alfalfa becomes more palatable to cattle as it approaches the bloom stage. Alfalfa in different fields at comparable stages of growth varied widely in palatability.

P22 Calf Losses in a Self-contained Herd Over a Period of Seventeen Years. R. E. JOHNSON, E. L. JUNGHIERR, AND W. N. PLASTRIDGE, Storrs Agricultural Experiment Station.

The data for this study were derived from post-mortem examination of 78 calves, representing a mortality of 7.24 per cent, which died prior to the age of 6 months in the University of Connecticut dairy herd during the 17-year period 1931 through 1947. Forty-one of these calves were males and 37 females. Each cadaver was subjected to pathological and bacteriological examination for diagnosis.

White scours, respiratory diseases, bloat and weakness at birth were the principal causes of death aside from miscellaneous conditions which accounted for 19 per cent of the mortality. White scours, responsible for 43.6 per cent of the losses, usually was observed during the first 6 days following birth; all but three of the 34 calves in this group died on or before the ninth day. The average age at time of death from white scours was 6.6 days. In a majority of cases, coliform organisms were isolated from either the cardiac blood, liver, spleen, navel artery or other internal organs. Cases showing gross evidence of navel infection occurred in this group. Respiratory conditions were responsible for 20.5 per cent of the losses. The average age at death in 16 cases was 47 days. Streptococci, staphylococci or pathogenic diphtheroids were isolated. Bloat was responsible for 9 per cent of the deaths, the ages of the calves ranging from 35 to 85 days. Weakness at birth accounted for 7.7 per cent of the deaths, which occurred at an average age of 6.2 days.

Although all calves up to 6 months of age were considered, no calves died after the first 93 days. There was no apparent seasonal effect on mortality when all causes of death were considered, but a slight tendency toward a higher incidence of white scours was observed during the period from August through January.

P23 The Effect of Prepartum Vitamin A Supplementation on the New-born Calf. A. A. SPIELMAN, H. D. EATON, J. K. LOOSLI, AND K. L. TURK, Cornell University.

Work has been done to determine the effect of supplementing the dry-cow ration with one million I.U. of vitamin A daily for 30 days prior to parturition on health and performance of the newborn calf. All cows received a commercial fitting grain mixture (12 per cent protein), mixed grass legume hay and corn silage. One group of 19 cows received only this standard dry-cow ration. Another group of 14 cows received the standard ration plus alfalfa leaf meal as a source of vitamin A. A third group of 9 cows received the standard ration and the alcohol form of vitamin A, while the fourth group of 16 cows received the standard ration

and the ester form of vitamin A. The calves from these cows remained with their dam for the first 2 days after birth. They were fed their mother's milk for the first week and herd milk thereafter. Calf starter, hay and water were fed *ad libitum*.

Calves from the dams fed the alcohol form of vitamin A were significantly higher in plasma carotenoids for the entire experimental period than calves from the other three groups. Calves from dams supplemented with the ester form of vitamin A were significantly higher in plasma carotenoids than those calves from dams fed alfalfa leaf meal. At any one age no significant differences were found between groupings. Calves within a grouping varied significantly among themselves during the experimental period and with age.

Calves from dams fed either form of vitamin A were significantly higher in the level of plasma vitamin A for the entire experimental period than calves from control dams or from dams fed alfalfa meal. There were no real differences between either group of calves from vitamin A-supplemented dams or between calves from control dams and calves from dams fed alfalfa. The significant differences cited above also existed at birth, 3 weeks of age, and 4 weeks of age. Calves within an experimental grouping showed significant differences in the blood plasma vitamin A levels with age and among themselves.

No statistical differences were found in the feed consumed by the various groups. Calves from dams fed either form of vitamin A were significantly heavier throughout the entire experimental period than calves from control dams or from dams fed alfalfa meal. In addition, calves from dams fed alfalfa meal were significantly heavier than calves from control dams. At any one age, the only statistical difference was at 4 weeks, when calves from dams fed the ester form of vitamin A were significantly heavier in liveweight than calves from control dams.

The calves from dams fed both forms of vitamin A had significantly less scours than calves from control dams. No real differences were found between calves from dams fed vitamin A and dams fed alfalfa meal.

P24 The Utilization of Fetal Liver Stores of Vitamin A by the Newborn Calf. A. A. SPIELMAN, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

Studies have been made to determine whether or not the vitamin A stored prenatally is utilized by the newborn calf. Calves from cows fed a standard dry-cow ration and calves from dams fed the same ration supplemented daily for 30 days prepartum with one million I.U. of vitamin A and 5 g. of soybean lecithin were removed from their dams at birth and fed reconstituted skim milk. Plasma carotene and vitamin A were determined

at birth and daily thereafter. The calves were slaughtered on the tenth day and the livers were analyzed for carotene and vitamin A.

At birth, calves from the vitamin A-supplemented cows had higher plasma vitamin A levels, which increased with age, whereas no such increase was noted in calves from the non-supplemented dams. At the end of 10 days the vitamin A remaining in the livers of the calves from vitamin A-supplemented cows was higher than the amount in the livers of the calves from non-supplemented cows. No appreciable differences between the two groups of calves were found in the carotene content of either plasma or liver.

P25 Effect of the Method of Administration of Carotene and of Vitamin A upon the Rate at Which They Are Absorbed from the Alimentary Tract of Dairy Calves. N. L. JACOBSON, G. H. WISE, AND R. S. ALLEN, Iowa State College.

At minimum intervals of about 1 week, carotene and vitamin A concentrates were added to the rations of young dairy calves to determine the effect of method of administration upon the rate of absorption of these substances from the alimentary tract. Milk, into which a vitamin supplement was homogenized, was fed to a series of paired individuals alternately by a nipple pail and by a stomach tube introduced into the rumino-reticular cavity. In a second series of calves, administration of the vitamin supplement by capsule was compared with nipple pail feeding of the homogenized product.

Results indicate that the rates of absorption, as measured by the concentrations of the carotene and vitamin A in the blood plasma, were more rapid when these substances pass directly to the abomasum than when they enter the rumino-reticular cavity. The absorption rate following administration of carotene in capsules indicated passage into the rumino-reticular cavity before entering the abomasum. The physiological significance of these findings has not been established, but it would seem possible that direct passage to the abomasum may enhance efficiency of utilization. The nature of the changes in blood plasma carotene and vitamin A levels following oral administration of carotene suggests a partial conversion of carotene to vitamin A in the intestinal wall.

P26 Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced by Ration Changes in Calves. W. C. JACOBSON AND J. W. THOMAS, Bureau of Dairy Industry, U. S. Department of Agriculture.

Weekly vitamin A determinations were made on 33 calves receiving a ration consisting of a limited quantity of whole milk to 60 days, a grain mixture and alfalfa hay. Six calves received 25,000 I.U. of vitamin A per day for the first 30 days; 12 received 50,000 I. U. of vitamin A per day for

varying periods of time; 15 received no supplemental vitamin A. At 90 days of age, all calves were placed on a vitamin A-deficient ration consisting of grain, skim milk, and wood shavings.

In general, there was a relationship between plasma vitamin A level and the vitamin A intake at these levels of supplementation. With this existing relationship it would seem that a reduction in the vitamin A intake by placing the calves on the deficient ration would cause a similar reduction in the level of plasma vitamin A. Instead, of 12 calves which received 50,000 I.U. per day, seven showed a decided increase in the vitamin A in the blood plasma. Two of the calves in the 25,000 I.U. group showed a slight increase, which persisted for 2 to 7 weeks. The plasma vitamin A level in the non-supplemented group gradually dropped to a low level when the calves were placed on the deficient ration. The reasons for the increase in the plasma vitamin A are not known. However, a relatively large store of vitamin A, along with the introduction of skim milk to the ration, may have been responsible for the increase in the plasma vitamin A levels.

P27 The Influence of the Ration on Some of the Blood Vitamin Changes in the Young Dairy Calf. J. W. HIBBS AND W. D. POUNDEN, Ohio Agricultural Experiment Station.

The plasma carotenoid level in calves fed whole milk and alfalfa hay from birth was observed to be markedly higher during the first 6 weeks than the levels in calves fed whole milk alone, whole milk plus grain, or whole milk plus grain plus alfalfa hay.

The inoculation of the rumens of the calves with rumen microorganisms from cows did not affect markedly the plasma carotenoids or vitamin A changes. The calves which had the highest plasma carotenoid levels maintained the lowest plasma vitamin A levels, indicating an inverse relationship between plasma carotene and vitamin A under these conditions. Liver storage probably complicates the plasma vitamin A level as a measure of vitamin A metabolism.

No marked variations were noted in the plasma ascorbic acid levels between groups fed whole milk and alfalfa hay and whole milk plus grain plus alfalfa hay. However, a higher, more uniform level of plasma ascorbic acid was maintained during the first 6 weeks in the calves which were inoculated with rumen microorganisms than in those not so inoculated.

These results, when correlated with those in paper P28, emphasize the value of good quality hay in meeting the vitamin needs of young calves through the establishment of early rumen function.

P28 The Influence of the Ration on the Digestive Tract Microorganisms of the Young Dairy Calf. W. D. POUNDEN AND J. W. HIBBS, Ohio Agricultural Experiment Station.

The development in the rumens of young calves of protozoa and bac-

teria of the types associated with alfalfa hay ingestion was helped by inoculation with microorganisms from rumens of mature stock, provided the calves were ingesting a sufficiently high proportion of hay. The numbers of protozoa increased as the proportion of grain increased in relation to the quantity of alfalfa hay ingested, until approximately equal parts of each were being consumed. Further increases in the proportion of grain were accompanied by reduction in numbers of both protozoa and hay-type flora until they eventually disappeared.

Bacteria of the types associated with grain ingestion made their appearance in appreciable numbers in samples once the proportion of grain consumed exceeded the hay; they continued to increase as the proportion of grain increased. Their development was not influenced by the inoculations. Indications are that the early development of rumen microorganisms similar to those observed in cows is influenced by the feeds consumed. In addition to the beneficial effect on certain blood vitamin constituents (see paper P27), the general appearance of the calves fed milk and hay alone was improved by rumen inoculation.

P29 Relation of Aerobic Bacterial Flora to the Consistency of the Feces.

M. D. VAN PELT, R. E. JOINSON, AND W. N. PLASTRIDGE, Storrs Agricultural Experiment Station.

The development of the bacterial fecal flora was followed in 26 calves through the first 19 days of life with special attention to *Escherichia coli*. The physical characteristics of the daily fecal samples were recorded with the rectal temperature of the calf. The total bacterial count and the gram-negative count were determined with differential media.

Calves are born with a sterile alimentary tract which rapidly becomes contaminated. The increase in bacteria is very great during the first 24 hours and, in calves raised according to a normal herd procedure, the flora tends to reach a peak on the second day. It then decreases slowly for 14 days, when it levels off. There is a seasonal difference in calves, with the fall calves having a significantly higher flora than summer calves.

The consistency of the feces goes through three phases: (a) the meconium, which is brown and elastic, lasting for 1 day; (b) the transitional stool, which is slimy in consistency and varies from light yellow to green in color, lasting about a week; and (c) the normal phase, which is dark brown, soft yet firm.

There was no significant correlation between high temperature or diarrhea and an abnormally high flora or a high ratio of *E. coli* to the total bacterial count.

Six calves were placed on skim milk at birth. All had periods of scouring and produced a transitional stool throughout the experimental period. Two calves died of white scours and *E. coli* was isolated from their liver,

kidney and spleen. During the actual periods of scouring, the flora was much lower than for previous samples. However, the flora was abnormally high 1.5 to 2 days before the scouring began. The flora of the calves fed skim milk was significantly higher throughout the period of observation than that of the normal calves.

P30 Raising Dairy Calves Without Colostrum. J. T. MILES, S. A. HINTON, AND HOMER PATRICK, Tennessee Agricultural Experiment Station.

Since prepartum milking is gaining in popularity as a means of relieving congestion in the cow's udder at calving time, and since valuable brood cows, because of disease or injury, may fail to produce utilizable colostrum at calving time, there is need for a method of raising calves without colostrum. Dairy bull calves born at the Knoxville Station were divided into three groups and fed as follows: Group I, dam's colostrum and milk for first week; Group II, a laxative at birth and herd milk fortified with vitamin A from birth to 1 week of age; Group III, a laxative at birth and herd milk fortified with a mixture of pure vitamins from birth to 1 week of age. All groups received the same kind of milk and other feeds after they reached the age of 7 days.

The six calves in Group I and eight calves in Group III have lived and made normal gains. Of the nine calves in Group II, four died before they reached the age of 30 days; others in this Group made subnormal gains.

P31 A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN, AND F. THORP, JR., Michigan State College.

Thirteen calves were divided into three groups and placed on synthetic milk diets at ages of 3 to 5 days. Group I received corn starch (dextrose), Group II dextrin, and Group III corn sugar as the principal carbohydrate source in the ration. Rations were identical except for the carbohydrate component. Twelve vitamins were added to the ration.

Weekly blood analysis showed normal red cell volume, hemoglobin, and magnesium but low levels of plasma calcium, inorganic phosphorus and ascorbic acid as compared to the values for the blood of similar calves on normal rations.

Growth was subnormal in all calves, indicating inadequacy of the ration. After 4 weeks on synthetic rations, the average change from the starting weight was +3.3 per cent, -8.8 per cent and -6.9 per cent for the sugar, dextrin, and starch groups, respectively. Calves on sugar showed a sleeker coat of hair and decidedly less tendency to scour than did calves of the

other two groups, but paralysis, curable by either potassium or biotin, affected all calves on sugar and none on dextrin or starch. The average survival time was 31.0; 16.6 and 31.3 days for the sugar, dextrin and starch groups, respectively; however, the average starting age was 13.7 days on starch as compared to 4.6 on dextrin and 3.8 on sugar.

Necropsy characteristically revealed no abnormalities outside the gastro-intestinal tract. Petechial hemorrhages in the abomasal mucosa were the most common finding; these were most numerous in the dextrin group but also appeared in the starch and sugar groups. Congestion of the duodenum and of the colon was prevalent in calves fed starch and dextrin but seldom was observed in calves on sugar.

P32 Effect of Tryptophan in the Diet on the Excretion of Niacin and Its Metabolic Products by Dairy Calves. G. C. ESH AND T. S. SUTTON, The Ohio State University and Ohio Agricultural Experiment Station.

Very young calves, fed a nicotinic acid-free ration, showed no deficiency symptoms (J. Biol. Chem., **167**: 729-736). Since the proteins of colostrum and milk are generously supplied with tryptophan, the possibility that dietary tryptophan may affect the urinary excretion of nicotinic acid and its metabolic products was investigated.

Five grams of L-tryptophan were fed daily for 3 days to each of two calves that had been maintained from birth on a whole milk diet. The urinary excretion of nicotinic acid, its metabolic products and tryptophan were determined on 24-hour samples and compared with similar data obtained previous to and following the tryptophan feeding.

The data indicate that N¹-methyl-nicotinamide is not the main metabolic product excreted by calves. Tryptophan feeding resulted in a 3-fold increase in the excretion of total free and combined nicotinic acid. There was little change in the excretion of free nicotinic acid and N¹-methyl-nicotinamide. The major portion of the increase was in the non-methylated products. The urinary excretion of tryptophan accounted for only 1 to 1.5 per cent of the intake. The data show that increases in dietary tryptophan result in increased urinary excretion of total nicotinic acid, indicating that tryptophan serves as a precursor of niacin in the calf as in other mammals thus far studied.

P33 Performance of Calves on a Photolyzed Milk Diet. R. G. WARNER AND T. S. SUTTON, The Ohio State University.

A recent report of riboflavin deficiency in calves fed a riboflavin-free synthetic milk (J. Nutrition, **33**: 263. 1947) prompted a study of the response of calves to a diet of milk in which the major part of the riboflavin

had been photolyzed. Approximately 96 per cent of the riboflavin in the milk fed was destroyed by exposure to the radiations of a 400 W mercury vapor lamp emitting light of wave lengths longer than 3000 Å. Four male Guernsey calves were fed the treated milk supplemented with vitamin A. One of these calves also received approximately 2.99 mg. of added riboflavin daily.

Deficiency symptoms consisted of intermittent diarrhea, a scurfy skin condition, alopecia, periodic excessive salivation and lacrimation, and, in the acute stages, difficulty in swallowing. These calves were extremely unthrifty. The addition of 2 mg. riboflavin daily to one of these calves resulted in a prompt cessation of diarrhea, resumption of growth, and marked improvement in general appearance, including the growth of new hair.

The performance of the calf receiving 2.99 mg. of added riboflavin from the start was uneventful and approached the Ragsdale standard in growth. Blood vitamin A and ascorbic acid levels were normal in all four calves. The urinary excretion of riboflavin varied between 0.01–0.06 mg. per day for calves receiving no added riboflavin and 0.38–0.64 mg. per day for the calf which received added riboflavin throughout the experiment. Data indicate that riboflavin requirement of a 70-lb. calf, is somewhat less than 3 mg. daily, when fed an exclusively whole milk diet.

P34 Anemia in Young Calves and Its Alleviation by Iron. W. C. JACOBSON AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

Anemia has been observed to occur in calves in the various herds at Beltsville. The anemia may be present at birth or may develop at any time up to 60 days of age. However, it usually corrects itself at about 80 or 90 days of age. About an equal percentage of calves from each of the breeds had hemoglobin values below 8 g. per 100 ml. except the Sindhi-Jersey crosses, which had a very high hemoglobin level for the first 90 days.

When a salt mixture of cupric sulfate, ferric sulfate, manganese chloride and cobalt sulfate was fed, the anemia was alleviated. When each of these minerals was fed separately, it was found that iron was the only one which alleviated the anemia. Red cell counts and red cell volumes were run along with the hemoglobin determinations on some of the calves receiving iron. After the calf had received iron for 2 weeks, there was an increase in the red cell count and an increase in the volume of the red cells as well as an increase in the quantity of hemoglobin in the blood. The weight gains to 90 days of age were studied. The calves with the lower hemoglobin values gained less on the average, but this difference was not statistically significant with the number of calves involved. However, the group of calves which had low hemoglobin values showed more variation in their weight gains.

P35 A Method of Evaluating Bull Semen. T. M. LUDWICK, D. OLDS, AND MARSHALL CARPENTER, Kentucky Agricultural Experiment Station and Kentucky Artificial Breeding Association.

Samples of diluted semen were incubated at 100° F. and observed under the microscope at regular intervals until motility ceased. Observations were made on data summarized from the Kentucky Artificial Breeding Association and cover a period of 11 months. The observations include 305 ejaculates from 27 different bulls of the Holstein, Jersey and Guernsey breeds from which approximately 12,000 cows were bred.

The coefficient of correlation between incubation time (time for sperm to lose all activity when held at 100° F.) and conception rate (based on 60-90 day non-returns) was 0.84 ± 0.03 when only ejaculates which were used in the breeding of as many as 30 or more cows were included. Ejaculates which were used to breed only a few cows did not give good correlations.

P36 Vital Staining of Bovine Spermatozoa with an Eosin-aniline Blue Staining Mixture. H. E. SHAFFER AND J. O. ALMQUIST, The Pennsylvania State College.

In 1942 Lasley, *et al.* (Anat. Record, 82: 167-174) reported upon an opal blue-eosin staining mixture for the differentiation of live and dead ram spermatozoa. Since opal blue was unobtainable, studies were undertaken in this laboratory to determine whether readily available dyes could be used. Aniline blue provided a suitable substitute for opal blue as the background stain. When used in combination with either eosin yellowish or eosin bluish, a satisfactory mixture for differential staining of bovine spermatozoa was obtained.

To determine the optimum concentrations of the dyes, seven levels of both eosin *Y* and eosin *B* (0.8 to 2.5 per cent) were tested in combination with 2, 3, 4 and 5 per cent aniline blue. The dyes were dissolved in a citric acid-disodium phosphate buffer, and five ejaculates of freshly collected bull semen were used to compare the staining properties of the 56 solutions. Four slides were prepared from each ejaculate using each of the staining solutions, and 100 sperm cells were counted per slide. Based on the quality and uniformity of the slides and the percentages of unstained spermatozoa (presumed to be living), 1 per cent eosin and 4 per cent aniline blue were selected.

Studies were conducted to determine the effects of concentration of buffer salts and pH of the buffer used to dissolve the dyes. Five concentrations of phosphate buffer (M/4 to M/12) were adjusted to pH 5.6, 6.4, 7.2 and 8.0, and 1.0 per cent eosin and 4.0 per cent aniline blue were added to each of the solutions. Differences in the percentages of unstained cells due to buffer concentrations were not statistically significant, while those due to

pH were significant at the 5 per cent level. When the appearance of the slides also was considered, a final staining mixture consisting of 1 per cent eosin B and 4 per cent aniline blue dissolved in M/8 phosphate buffer having a pH of 7.2 was selected as a satisfactory differential stain for bull spermatozoa.

A simple technique for preparing uniform slides has been developed. The slides are dried on a hotplate maintained at a temperature of 85 to 100° C. and an electric fan is used to hasten the drying process. Field trials now are in progress to determine the value of this staining method as a measure of semen quality.

P37 Turbidometric Assay of Hyaluronidase in Bull Semen. JOHN P. MIXNER AND JAMES E. JOHNSTON, New Jersey Agricultural Experiment Station.

Hyaluronidase (*h*-ase) is an enzyme found in bull semen which has the ability to depolymerize hyaluronic acid. Hyaluronic acid is an important constituent of tissue cell cement. The turbidometric assay for *h*-ase is based on the discovery that the turbidity produced by the interaction of hyaluronic acid and acidified blood serum is, within limits, a function of the hyaluronic acid concentration. The conventional unit of *h*-ase, the turbidity reducing unit (TRU), is not comparable between laboratories due to variations in serum protein concentrations, hyaluronic acid preparations and pH control in assaying. Comparative assaying of *h*-ase is greatly facilitated by assaying in terms of a standard preparation of *h*-ase which may be exchanged among laboratories. The coefficient of correlation secured between milligrams of standard *h*-ase and colorimeter reading was -0.985 ± 0.006 . The standard error of estimate of the regression equation gives an error of ± 8 per cent on the mean value of milligrams of *h*-ase.

H-ase assay values of semen in terms of milligrams of standard *h*-ase, secured by diluting semen at various rates, conformed to the standard regression line of purified *h*-ase. This is important since the concentration of salts, particularly NaCl, influences greatly the activity of *h*-ase. The data for 117 semen assays were calculated both in TRU's and in milligrams of standard *h*-ase. A coefficient of correlation of $+0.954 \pm 0.028$ was secured between the two, indicating the essential sameness of the two measures of *h*-ase potency.

P38 Hyaluronidase and Bull Semen. J. E. JOHNSTON, E. J. STONE, AND J. P. MIXNER, New Jersey Agricultural Experiment Station.

The enzyme hyaluronidase (*h*-ase) is capable of denuding the mammalian ovum of follicular cells adhering after ovulation. Therefore, a relationship between *h*-ase and fertility has been postulated.

An investigation of bull semen in relation to *h*-ase has shown: (a) that *h*-ase is present immediately after ejaculation, (b) that the concentration of *h*-ase in the seminal plasma increases on incubation of the semen at either 5 or 37° C., (c) that a maximum potential concentration of *h*-ase is obtained on incubation of semen for 24 hours at 37° C. under toluene, and (d) that *h*-ase seems to be tied up with the spermatozoa, since incubation of sperm-free seminal plasma yields no increase in *h*-ase concentration. On this basis, procedures for determining semen *h*-ase potency were adopted in which semen samples were assayed initially, within an hour after ejaculation, and again after 24 hours incubation at 37° C. under toluene.

One hundred semen samples were analyzed; a coefficient of correlation of $+0.639 \pm 0.060$ was obtained between initial and 24-hour *h*-ase concentrations. The following coefficients of correlation were obtained between initial *h*-ase concentrations and other semen characteristics: sperm concentration per mm³, $+0.540 \pm 0.072$; initial motility, $+0.0425 \pm 0.101$; volume of ejaculate, $+0.157 \pm 0.095$; sperm per ejaculate, $+0.479 \pm 0.078$; and duration of second motility, $+0.0132 \pm 0.101$. Twenty-four-hour *h*-ase concentrations gave the following coefficients of correlation: sperm concentration per mm³, $+0.697 \pm 0.052$; initial motility, $+0.245 \pm 0.095$; volume of ejaculate, $+0.206 \pm 0.097$; sperm per ejaculate, $+0.540 \pm 0.072$; and duration of second motility, $+0.0018 \pm 0.101$.

P39 The Effect of Testis Biopsy on Semen Characteristics. J. F. SYKES, W. J. SWEETMAN, P. C. UNDERWOOD, AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

A marked reduction in sperm concentration resulted when testis biopsies were performed on three bulls. Two biopsies were performed on each animal, and a progressive drop in sperm concentration occurred with succeeding biopsies in every instance. The first biopsy had little or no effect on other sperm characteristics, but after a second biopsy both initial and storage motility were reduced.

Microscopic examination indicated that widespread damage occurs in the germinal epithelium following biopsy. Repair appeared to occur, but in spite of this apparent recovery there was no increase in sperm concentration even after intervals of 6 to 15 months.

P40 Spermatozoa Behavior in Bovine Cervical Mucus at Varying Stages of Estrus.¹ H. A. HERMAN AND OTIS H. HORTON, University of Missouri.

To gain additional information on factors affecting the conception rate in dairy cattle, a study of the mucus secretions of the cervix and uterus has

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1109.

been undertaken. The viscosity and penetrability of mucus are factors in spermatozoa movement through the uterus and up the tubes. The consistency of mucus changes during the estrus period. In early heat it is thin and watery; as heat progresses it becomes more viscous and there is an infiltration of leucocytes.

The properties of cervical and vaginal mucus were studied with respect to flow-elasticity, penetrability of spermatozoa, volume, pH and leucocyte number. Vaginal temperatures also were recorded. The viscosity of cervical and vaginal mucus was found to be lowest during the first 6 hours of estrus and gradually increased as estrus was prolonged. Penetrability of the mucus by spermatozoa was highest during the first 6 to 10 hours of heat. The volume of mucus was greatest during the first part of the heat cycle. There was a rise in vaginal temperatures during estrus, the peak temperature generally occurring 14 to 16 hours after the beginning of the heat period. The mean pH of mucus *in vivo* was found to be 7.23 during estrus and ranged from 7.25 to 7.39 during non-estrus.

The results in these preliminary studies indicate that the properties of bovine mucus may be of considerable interest in determining the most satisfactory practices to follow in obtaining a high conception rate where artificial insemination is used.

P41 Varying the Proportion of Egg Yolk in Diluters for Bull Semen.

ERIC W. SWANSON, University of Tennessee.

A study was made of the sodium citrate and egg yolk semen diluter to establish optimum concentrations of the respective constituents and to detect the minimum amount of egg yolk which will produce satisfactory results. Concentrations of 1, 2, 3, 4 and 5 per cent of sodium citrate were compared in the egg yolk diluter to determine the least harmful citrate concentration. Concentrations of 2 and 3 per cent were satisfactory, with the latter being slightly superior. This solution also was most nearly isotonic with bull semen. Diluters were prepared with egg yolk and 3 per cent sodium citrate mixed in proportions of 1 to 1, 1 to 3, and 1 to 7. Daily estimations of percentage progressively motile sperm were made on semen diluted identically with the three diluters. Very little difference was noted between the 1 to 1 and 1 to 3 diluters, and the 1 to 7 diluter was very satisfactory although slightly inferior. The ability of freshly collected sperm to withstand temperature shock in the three diluters was checked by means of differential stain for dead and alive sperm before and after shock. The differences between the diluters were small and of doubtful significance. Therefore, a diluter containing one-eighth egg yolk may be used with the advantage of economy of egg yolk and of time and easier microscopic examination.

P42 A Study of the Types of Bacteria in Bovine Semen and Their Effect Upon Motility.¹ J. E. EDMONDSON, K. L. TALLMAN, AND H. A. HERMAN, University of Missouri.

Since most semen, either diluted or non-diluted, contains variable concentrations of bacteria, the authors have been interested in securing more detailed information regarding the effect of bacteria on the length of storage of bovine semen. The early work consisted of isolating the different types of organisms found in semen. The types isolated included streptococci, staphylococci, micrococci, pseudomonas, bacilli, actinomyces, yeast and *Escherichia coli*. Standard plate counts were made of both diluted and non-diluted semen to determine if there was any correlation between the number of organisms present and the length of time semen could be stored. Careful analysis of the data showed no correlation; however, when samples were plated on blood agar, a definite correlation existed between the number of hemolytic organisms and the length of storage of semen. Using motility as the index of storage, the hemolytic count was found to increase as the length of storage decreased.

The effect of hemolytic and non-hemolytic bacteria upon the length of storage of semen was further proved by studies using fresh diluted and non-diluted semen to which was added a pure culture of organisms previously isolated from semen. Semen samples inoculated with hemolytic bacteria showed no motility after 2 days of storage, while samples containing non-hemolytic bacteria varied in length of storage. Certain non-hemolytic organisms were able to increase the storage time from 1 to 4 days over the controls, while others did not store as long as the controls. It is believed that certain organisms are able to increase storage time of semen either by enzymatic action or by entering into the metabolic processes of the sperm.

P43 The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. JOHN O. ALMQUIST, The Pennsylvania State College.

Previous studies at this Station showed that penicillin inhibited bacterial growth in semen but did not significantly affect the fertility of semen from bulls of relatively high breeding efficiency. To test the effect of penicillin upon the fertility of semen from relatively infertile bulls used in routine artificial breeding, a 5 x 5 Latin square experiment was designed and replicated four times in succession. Penicillin was added to the semen of four Guernsey bulls and one Holstein bull of lowered fertility at the rate of 250, 500, 750 and 1,000 units per ml. of diluter semen with appropriate controls. Data based on 6-month non-returns for 3,576 first and second services demonstrated that the 500- and 1,000-unit levels of penicillin brought

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1106.

about highly significant increases in fertility when compared to the untreated controls. Greatest improvement in fertility was obtained with the 1,000-unit level, but the degree of enhancement varied considerably between bulls. Three of the five bulls showed average increases above the controls of 15, 21, and 31 per cent on the basis of 6-month non-returns when penicillin was added at the rate of 1,000 units per ml. of diluted semen. The remaining two bulls showed small increases of 1 and 4 per cent. Apparently only three of the bulls produced semen in which bacteriological control by penicillin was beneficial. The 13-year-old bull which showed the 1 per cent increase was slaughtered because of low fertility 7 months after the close of the experiment. Histological examination revealed marked degeneration of the testes. The untreated semen of the bull which showed a 4 per cent response increased in fertility to such a degree during the experiment that the animal could not be considered a problem bull.

P44 Breeding Results With Bovine Semen Treated With Varying Amounts of Thyroxine. A. B. SCHULTZE AND H. P. DAVIS, University of Nebraska.

Semen samples from 22 bulls, selected at random, were treated with crystalline *d,l*-thyroxine. The relative fertility of this semen was determined from breeding results in artificial breeding associations from the percentage of non-return services after a 4-month period. The conception rate obtained with other semen samples from the same bulls during the same period served as a basis of comparison. Thyroxine was added in the amounts of 2 γ , 10 γ , and 15 γ per 100 ml. diluted semen. The 2- γ level resulted in an increase of 2.2 percentage units in conception rate (732 services, 23 semen samples). The 10- γ level in an increase of 6.4 percentage units (1,847 services, 112 semen samples); and the 15- γ level in an increase of 4.4 percentage units (488 services, 18 semen samples).

P45 Measuring Breeding Efficiency by Pregnancy Examinations and by Non-returns. G. R. BARRETT, L. E. CASIDA, AND C. A. LLOYD, University of Wisconsin.

This report includes data on inseminations made during 1946 in the Wisconsin Experimental Breeding Project, in the area in which routine pregnancy examinations were performed at 35-49 days after breeding. Pregnancy examination information was available for 7,530 inseminations. Of the 4,286 inseminations reported as first services, 52.9 per cent resulted in pregnancies, as determined by pregnancy diagnosis. The 30-60 day non-return percentage for these same inseminations was 67.8, the 60-90 day percentage was 58.4, and the 90-120 day figure was 55.7 per cent. Thus, the discrepancies in favor of the non-return percentages were 14.9, 5.5 and 2.8 per cent. Data concerning services other than the first also will be pre-

sented, as well as a study of monthly variation in the conception and non-return rates.

P46 Order Number of Insemination and Conception Rate. G. R. BARRETT, C. A. LLOYD, AND R. A. CARPENTER, University of Wisconsin.

Inseminations performed in the Wisconsin Experimental Breeding Project from January 24 to May 31, 1945, and from August 13, 1945, to Sept. 30, 1947, were summarized. Pregnancy examinations were performed, usually 35 to 49 days after insemination. An insemination was considered fertile only when an amniotic vesicle could be palpated; inseminations were considered infertile if the cow was rebred or if she was diagnosed not pregnant. Inseminations were excluded if the cow had died, been sold, or could not be examined for any other reason. Of 14,771 inseminations, the number in each group from first to seventh service was 8,621, 3,463, 1,443, 631, 308, 152, and 75, respectively. There were 78 inseminations with order numbers higher than seven. The corresponding percentages of inseminations classed as fertile were 54.4, 50.7, 46.5, 38.2, 36.7, 30.1, 27.7 and 16.7. The mean percentage of fertility for the entire 14,771 inseminations was 51.1.

P47 The Effect of Udder Inflation of Cows with Parturient Paresis, on Blood Calcium, Magnesium and Inorganic Phosphorus. VEARL R. SMITH AND R. P. NIEDERMEIER, University of Wisconsin.

The udders of five Jersey cows¹ in advanced stages of parturient paresis (down and unable to rise) were inflated with air to a pressure of 60 to 70 mm. mercury. A pre-inflation venous blood sample was taken, and post-inflation samples were taken at 0.5, 1.5, 3, 5, 8, 11, 14, 17, 20, 36 and 48 hours. Samples were analyzed for total serum calcium, serum magnesium and plasma phosphorus. Pre-inflation blood calcium, inorganic phosphorus and magnesium ranged from 5.2 to 4.4, 1.4 to 0.5, and 4.5 to 2.0 mg. per cent, respectively. The cows arose of their own volition from 3 to 14 hours after inflation; with one exception, the blood serum calcium was 6 mg. per cent or above at the time the cow arose. The normal phosphorus level had not been reached in four of the five cows by the twentieth hour post-inflation. Three of the five cows had high magnesium levels through the twentieth hour post-inflation.

P48 A Study of Citric Acid Levels in the Blood and Urine of Cows at Time of Parturition: T. H. BLOSSER AND VEARL R. SMITH, University of Wisconsin.

The importance of citric acid in calcium metabolism has been shown by

¹ Four of these cows are in the herd of Biltmore Farms, Biltmore, North Carolina. The authors are grateful to the management of Biltmore Farms for making these animals available for the study herein reported.

several workers. Studies have been undertaken to study the relationship of citric acid to the changes that occur in the blood calcium levels at time of parturition. Studies on the blood citric acid and calcium of 18 cows prior, during and subsequent to parturition in general show that as the blood calcium decreases, there also is a decrease in the citric acid. Those cows having parturient paresis at parturition showed much lower levels of blood citric acid than normal cows. Preliminary studies show that there is an increased excretion of citric acid prior to parturition in cows having parturient paresis.

P49 The Effect of Prepartum Milking on Some Blood Constituents of the Cow. R. E. JOHNSON, H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

Two groups of cows, one milked twice daily 10 days prior to the calculated parturition date and the other milked shortly after calving, were used in this experiment. Total hemoglobin, plasma carotene and vitamin A, serum calcium and phosphorus were determined on venous blood samples drawn at weekly intervals beginning 4 weeks before calving, immediately following calving and ending 4 weeks after parturition. In addition, mammary and umbilical edema, expulsion of the placenta, and incidence of clinical milk fever and ketosis were recorded.

No apparent differences in blood constituents were noted between cows milked prepartum and postpartum. Total hemoglobin increased slightly at parturition and thereafter decreased to a slightly lower level than that obtained during the 4-week prepartum period. Both plasma carotene and vitamin A showed a marked drop at the time of parturition and increased thereafter, but they did not attain the same level as that found prepartum. Serum calcium and serum phosphorous dropped appreciably at parturition and returned to the prepartum level within a week after parturition.

To date, due to the limited observations, no significant conclusions can be drawn with regard to the effect of prepartum milking on mammary and umbilical edema, expulsion of the placenta and incidence of ketosis and milk fever.

P50 A Study of Some Blood Constituents of Cows not Milked Following Parturition. R. P. NIEDERMEIER AND VEARL R. SMITH, University of Wisconsin.

Four Jersey cows, not first-calf heifers, were used for this study. Venous blood samples were taken for 5 days previous to the anticipated day of parturition and for 8 days following calving, or until pressure in the udder had subsided. Calves were removed from their dams before being permitted to suckle, and no milk was removed from the udder. Twice-a-

day milking began the seventh day after parturition in one cow, but she never attained a full flow as judged by previous lactations. The other three cows never were milked and later were mastectomized. Blood samples were analyzed for total serum calcium. All four cows showed an appreciable drop in blood calcium on the day of parturition. One cow had parturient paresis 36 hours postpartum. The blood calcium level returned to normal levels by the second day postpartum. Higher-than-normal levels of blood calcium occurred on the third or fourth day postpartum, which seemed to be the time of greatest intramammary pressure.

P51 The Effect of Preparturient Milking on the Composition of Colostrum. A. H. VAN LANDINGHAM, C. E. WEAKLEY, JR., R. A. ACKERMAN, AND GEORGE HYATT, JR., West Virginia Agricultural Experiment Station.

A group of 11 cows and heifers were milked from 3 to 18 days before calving. Samples of preparturient colostrum were taken for chemical analysis when as much as 2 lb. per day was obtained. Total nitrogen, non-casein nitrogen, casein nitrogen by difference, and fat were determined. Samples also were obtained from a comparable group of 11 cows and heifers which had not been milked prepartum.

Colostrum obtained from heifers not previously milked on the day of calving contained an average of 2.34 g. per 100 ml. of total nitrogen, of which 57.3 per cent was in the non-casein nitrogen fraction. By the end of the fourth day following parturition, the total nitrogen was reduced to 0.70 g. per 100 ml., of which only 20.8 per cent was in the non-casein fraction.

The total production of preparturient colostrum varied within the 11 animals from 2 to 142 lb. The total nitrogen and the proportion of non-casein nitrogen to total nitrogen on the day of calving were related to the amount of preparturient colostrum produced. The two heifers producing the most preparturient colostrum secreted colostrum very similar in composition to that of normal milk 1 to 3 days before calving. Other heifers which produced only small amounts of preparturient colostrum before calving produced colostrum after calving very similar in composition to that of heifers which had not been pre-parturient milked.

P52 The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

A comparison of the carotene, vitamin A, and proximate composition of colostrum of two groups of cows has been made. One group was milked

twice daily for 10 days prior to the calculated parturition date; the other group was not milked until after calving. Calves born to these cows were not allowed to nurse. Pooled aliquot portions of the morning and evening milking for each day prepartum and samples from the first six milkings postpartum were analyzed for carotene, vitamin A, protein, lactose, fat and ash.

The first milking postpartum from prepartum-milked cows was similar in composition to normal milk, especially in those cows milked for at least 10 days before calving. The first milking after calving from postpartum-milked cows contained approximately five times as much carotene and vitamin A, three to four times as much protein, one-half as much lactose, slightly greater amounts of fat, and one and one-fourth times as much ash as the milk obtained from the prepartum-milked cows. These values decreased with successive milkings. Prepartum milking materially alters the composition of the first milk secreted at the termination of pregnancy and produces a milk much lower in nutritive value, as indicated by the analyses.

P53 The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, R. E. JOHNSON, AND R. J. SLATE, University of Connecticut.

The usual practice is to allow the calf to nurse immediately after parturition. In many cases the cow then is partially milked out. The problem was to establish the relative nutritive value of the successive portions of this first colostrum.

Immediately after calving, cows were completely milked out by 2-lb. increments. The carotene and vitamin A, protein, lactose, fat and ash contents were determined on these samples. Carotene and vitamin A and fat increased with successive increments, while lactose and ash decreased and protein remained essentially the same. Cows milked prepartum and cows milked postpartum gave essentially the same trends.

P54 Effect of the Form of Vitamin A and of Tocopherol Supplements of the Ration on the Concentration of Vitamin A and Carotenoids of Colostrum and Early Milk. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES, Kansas Agricultural Experiment Station.

Determinations were made of vitamin A and carotenoid concentrations of colostrum and early milk from groups of cows that received either a basal ration or a basal ration plus vitamin supplements during the terminal month of gestation. The supplements used were: (a) 0.5-1 million units vitamin A ester, (b) 0.5-1 million units vitamin A alcohol, (c) 0.5-1 million units of vitamin A alcohol plus 0.5-1 g. tocopherols, (d) from 0.5-1 g. to 10 g. of tocopherols.

Wide individual differences were noted in vitamin A and carotenoid contents of colostrum and early milk from cows receiving the various supplements. Consistent differences in the levels of vitamin A did not result from supplementation of the ration with the ester and alcoholic forms of vitamin A. No increase was noted in the vitamin A of colostrum and early milk from cows that received tocopherols in addition to the alcoholic form of vitamin A. Data suggested that tocopherol supplements at high levels might have increased the vitamin A content of colostrum, but due to the small number of animals used, the latter effect was not definitely established. The various supplements had no apparent effect on carotenoid contents of colostrum and early milk.

P55 Comparison of Barn-cured and Field-cured Alfalfa Hay. GILBERT H. ROLLINS AND PAUL M. REAVES, Virginia Polytechnic Institute.

Alfalfa hay was divided into two lots in the field by using alternate windrows. One lot was placed on a barn hay drier when partially cured and curing completed by forced atmospheric air. The other lot was allowed to cure in the field. A double reversal feeding trial was conducted to compare the milk-producing ability of the two hays. Each period was 28 days in length, with a 3-day changeover period. Hay was fed as the sole roughage and grain was fed according to milk production. Twelve cows were used in the trial.

The production of 4 per cent fat-corrected milk was 15,573 lb. while the cows were on the barn-cured hay and 14,994 lb. while on the field-cured hay, or approximately 4 per cent more milk for the barn-cured hay. The decrease in production was more pronounced from the field-cured hay as the trial progressed.

The carotene content of the hay after storage was approximately 60 per cent higher for the barn-cured hay. It was rated as having 62 per cent color and 45 per cent leafiness compared to 47 per cent color and 35 per cent leafiness for the field-cured hay.

P56 Studies on Mow Curing of Baled Hay. W. A. KING, J. W. WILBUR, S. M. HAUGE, AND A. W. COOPER, Purdue University.

Field-cured and mow-cured baled alfalfa hay have been compared in feeding trials for a 2-year period. Unheated air was used to finish the drying of the mow cured hay, which ranged from 30 to 35 per cent moisture when placed in the mow. All the hay was grown in the same field and alternating windrows were used for the field and mow curing. The first-cutting hays contained considerable timothy. Each of the three cuttings of each year were fed to dairy cows during a test period of 9 weeks. The carotene contents of the hays as fed for each of the 2 years were as

follows: first cutting, field—10 and 3 parts per million, mow—13 and 7 p.p.m.; second cutting, field—9 and 2 p.p.m., mow—25 and 15 p.p.m.; third cutting, field—25 and 12 p.p.m., mow—25 and 39 p.p.m. Feeding trials using six cows in each group showed no consistent results in favor of either method of curing. The hay was fed at the rate of 2.5 lb. per 100 lb. of liveweight. It was found that 25 p.p.m. of carotene in the hay would maintain the carotene content of the blood plasma and milk at only a fair level. Hays of 39 p.p.m. of carotene or more are necessary for the maintenance of good levels of carotene in blood and milk. In the mow curing of baled hay, unheated air apparently cannot be depended upon regularly to produce a superior product.

P57 Stack Finishing of Baled Hay with and without Heat. K. A. KENDALL, W. B. NEVENS, AND J. H. RAMSER, University of Illinois.

Numerous trials in the finish curing of long, chopped, and baled hay by means of a mow ventilation system have shown this method of curing to be advantageous as compared with field curing. Six trials in the finish curing of baled hay were carried out, two of them with supplemental heat supplied by oil-burning units. Stacks ranging in size from 135 to 300 bales were constructed with a central duct formed by bales.

The moisture content of hay as baled should be no higher than for finish curing in other forms. Baled hay with 47 per cent moisture could not be dried even with supplemental heat. Hay baled with about 30 per cent moisture was successfully dried. Samples showed 15.7 per cent protein and 21,000 units carotene per pound (average values). Eleven gallons of fuel oil and two gallons of gasoline were used per ton of dry hay. Bales tightly tied could not be dried successfully. Small, loose bales are needed. All bales must be stacked on edge and those next the duct spaced 1 to 2 inches apart to permit circulation of air. The outside bales must be placed close together and tarpaulins kept tightly tied over the stack to aid air circulation. Supplemental heat permits drying at night and during rainy weather.

P58 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and a Poor Quality Field-cured Hay. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, C. G. MELIN, W. J. SWEETMAN, W. H. HOSTERMAN, AND H. M. TYSDAL, Bureau of Dairy Industry; Bureau of Plant Industry, Soils, and Agricultural Engineering; of Agricultural Research Administration; and Hay Section, Grain Branch, Production and Marketing Administration. United States Department of Agriculture.

Cooperative forage harvesting and feeding experiments were conducted

at Beltsville for the third year during 1947. In contrast with previous years, unfavorable weather occurred during harvest and the field-cured hay was badly damaged by rain. Therefore, the field-cured hay was of poor quality while the silage and barn-cured hay were of good quality. The second cutting alfalfa crop used was variable and quite weedy. The silage and barn dried hays were harvested with a field chopper. Supplemental heat was used in barn hay drying.

Nutrient preservation in the wilted alfalfa silage, barn-dried hay and field-cured hay was, respectively: dry matter, 86, 91 and 60 per cent; protein, 83, 83 and 49 per cent; carotene, 3.6, 5.3 and 0.6 per cent. The labor and equipment requirements were about the same for the silage and barn-dried hay but were much higher for the field-cured hay due to large field losses.

Average daily milk production per cow on wilted silage, barn-dried hay and field-cured hay was 37.1, 36.2 and 35.2 lb., respectively, and the 30-day declines in production were 7.7, 8.8 and 13.6 per cent. On an acre basis (including other feeds), milk production was 40 per cent higher on the silage and 48 per cent higher on the barn-dried hay than on the field-cured hay.

P59 Vitamin D Content of Forages as Affected by Various Curing Procedures. J. W. THOMAS AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

The vitamin D content, expressed in International Units per pound of air-dry material, of an alfalfa crop harvested in 1945 as wilted silage, barn-dried hay and field-cured hay, was 254, 213 and 440, respectively. Values of 393, 264 and 400, respectively, were obtained for alfalfa harvested in 1946.

Alfalfa brought into the barn for mow drying immediately after cutting and at three stages of maturity was found to contain appreciable amounts of vitamin D. The alfalfa cut at the mature or seed stage contained much more vitamin D than that cut at half-bloom. The latter contained slightly more than that cut at bud stage. All three hays supplied sufficient vitamin D to prevent any visible signs of rickets in calves when the hays were fed as the only source of vitamin D to calves kept in a darkened barn from birth to 8 months of age.

The amount of dead leaves adhering to the plant at cutting time of the bud, half-bloom and mature stages was 2.4, 2.9 and 6.5 per cent, respectively. These dead leaves contained approximately 3,200 I.U. of vitamin D per pound of air-dry material. No vitamin D was detected in totally green leaves. Thus, the amount of dead leaves adhering to the harvested forage plant may modify considerably the vitamin D content of forages irrespective of length of exposure to sunlight during curing.

P60 Comparison of Early-cut and Late-cut Lespedeza Hay for Milk Production. C. E. WYLIE, J. A. EWING, ERIC W. SWANSON, AND J. M. MADDUX, University of Tennessee.

The differences in feeding value of Korean lespedeza hay cut in the bloom stage and in full seed stage have been measured with milk cows in five winter feeding trials. In the first four trials, groups of six cows each were fed *ad libitum* early- and late-cut lespedeza hay, respectively. They also were fed 10 lb. of corn silage daily and concentrates according to production. The early-cut hay was more palatable, was consumed in larger quantities, and resulted in more milk production. The average production per ton of hay fed was 109 lb. 4 per cent fat-corrected milk more for the early-cut hay. The early-cut hay gave a greater return per ton in three of the four *ad libitum* trials. In the fifth trial, lespedeza hay was the sole roughage ration and grain was fed at a wider ratio to milk production. Carefully paired milking cows were placed in two groups of five and fed so that hay consumption was equal. The average daily production per cow was 3.58 lb. of 4 per cent milk higher on the early-cut hay, with a progressive increase in difference between the two groups as the trial continued. The average fortnightly decline was 0.50 lb. 4 per cent milk in the group fed early-cut hay and 0.72 lb. in the group fed late-cut hay.

P61 The Influence of Various Hays on the Production, Vitamin Content, and Flavor of Milk. J. K. LOOLSI, V. N. KRUKOVSKY, AND G. P. LOFGREEN, Cornell University.

Fifteen Holstein cows were fed six types of hay in an incomplete block design experiment involving four periods of 5 weeks each. The hays studied included early-cut timothy, late-cut timothy, second crop alfalfa cut at early and late stages of maturity, birdsfoot trifol (*Lotus corniculatus*) and ladino clover. Measurements were made of the palatability of the hays and of their effects upon milk production and on the carotene, vitamin A and tocopherol contents and flavor of the milk.

The late-cut timothy proved much less palatable and resulted in a lower milk production than any of the other hays. On *ad libitum* feeding the average intake of late-cut timothy was only 35 to 44 per cent as much as of the other hays, and the actual milk production was approximately 25 per cent lower.

The milk produced when birdsfoot trifol was fed was appreciably higher in carotene, vitamin A and tocopherol content than from any other hay. The milk produced during the periods when ladino clover and late-cut timothy were fed was lower in these vitamins than when early-cut timothy or alfalfa hay was fed. Milk of poor keeping quality resulted during ladino feeding and could be correlated with the low contents of vitamin A, carotene and tocopherol. The study will be repeated to obtain more infor-

mation concerning the influence of hay upon the fat soluble vitamin content and the keeping qualities of the milk.

P62 Comparison of Digestion Coefficients of Sun-cured and Barn-cured Hays from the Same Field. O. M. CAMBURN, Vermont Agricultural Experiment Station.

This report covers three crop years, 1944-1946, for which there were, respectively, two, four and three pairs of hays. From each field, after partial field curing, one portion was placed on flues in the barn to be finished. Another portion was sun cured and then stored to sweat-out in the mow.

Each year the barn-cured hay, as fed, carried more crude protein and nitrogen-free extract than the sun-cured hay which was higher in crude fiber content. The ether extract contents were similar. For barn-cured hay, the digestion coefficients were higher for crude protein for 2 years and higher every year for nitrogen-free extract. For sun-cured hay, the crude fiber coefficients were higher every year; ether extracts were higher for 2 years. The digestible protein was higher for barn-cured hay for 2 years and identical 1 year; for digestible nitrogen-free extract, the barn-cured hay excelled for 3 years; digestible crude fiber of the sun-cured hay was higher for 3 years and ether extract for 2 years. When the results for 3 years are averaged, the digestible protein and total digestible nutrients are similar for hays cured by these two methods.

P63 Lactating Factors for Dairy Cows in Dried Grapefruit Peel. R. N. DAVIS AND A. R. KEMMERER, University of Arizona.

Eight cows—four Guernseys, two Jerseys, and two Holsteins—were fed alfalfa hay *ad libitum* until milk production definitely decreased. At the end of this period the ration was supplemented daily with 4 lb. of dried grapefruit peel for 5 weeks. Then the dried citrus was replaced with an equal amount of a mixture consisting of rolled barley 6 parts, wheat bran 6, cottonseed meal 2, and beet pulp 2. After 4 weeks this ration was supplemented with oat pasture. Four pounds of dried grapefruit peel added daily to an alfalfa hay ration increased milk production. An equal amount of a grain mixture did not maintain this increase. Supplementing a ration of alfalfa hay and concentrate mixture with oat pasture definitely increased milk production. Dried grapefruit peel contains factors which stimulate milk production in dairy cows.

P65 The Growth of Dairy Heifers Reared on Maximum Roughage with Varying Amounts of Grain.¹ O. T. STALLCUP, H. A. HERMAN, AND A. C. RAGSDALE, University of Missouri.

Feed consumption and growth of Holstein heifers on rations utilizing

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1112.

hay, silage and roughage to a maximum extent, together with minimum amounts of grain, have been recorded. Three trials were conducted in which the average consumption of feedstuffs and pasture from 6 to 24 months of age was as follows: (a) 796 lb. grain, 1728 lb. lespedeza hay, 115 lb. alfalfa hay, 2265 lb. sorgo silage, and 334 days on pasture; (b) 912 lb. grain, 3934 lb. lespedeza hay, 3757 lb. sorgo silage, and 262 days on pasture; (c) 1232 lb. grain, 1870 lb. lespedeza hay, 773 lb. alfalfa hay, 1975 lb. mixed clover and grass hay, and 263 days on pasture.

The mean body weight of all groups was slightly below normal standards at 24 months of age. The height at withers was only slightly below normal for all groups. The circumference of chest was slightly above normal in the first group and slightly below normal in the other groups. A fourth group of heifers fed on hay, silage, pasture and minerals from 15 to 24 months of age was slightly below normal in weight at 24 months. None of the deviations of the groups from normal, either in body weight or in body measurements, was statistically significant. It is concluded that heifers may be grown satisfactorily from 6 to 24 months of age on rations utilizing hay, silage and pasture to the maximum with but little sacrifice in growth.

P66 Wintering Dairy Heifers on Legume Hay. S. A. HINTON, J. T. MILES, AND C. E. WYLIE, Tennessee Agricultural Experiment Station.

Results of trials at Knoxville show that dairy heifers over 1 year of age can be well grown without concentrate feeding if good pastures are provided in the spring, summer and fall and if ample amounts of legume hay are fed during the winter. The trials included comparisons of field-cured and air-dried hay as winter feed for heifers. Sixteen yearling dairy heifers pastured on permanent pasture through the summer and fall were brought into the barn on December 1. They were divided as equally as possible into two groups, one group being fed field-cured long hay and the other bin-dried chopped hay. No concentrates were fed. Salt and minerals were kept before them at all times.

Heifers fed both kinds of hay made normal gains, averaging 1.38 lb. per day per heifer for those on field-dried hay and 1.33 lb. when fed bin-dried hay. Heifers ate an average of 25.7 lb. each daily of the field-cured hay and 24.2 of the bin-dried hay. Holstein heifers made much greater gains on the hay feeding than did the Jerseys; however, when they were put on pasture after the hay feeding period, the Jerseys gained as rapidly as did the Holsteins. Heifers in both groups and of both breeds gained approximately 1 lb. each daily on pasture.

P67 Observations on Calves Dehorned with an Antimony Trichloride-salicylic Acid-collodion Preparation. G. E. STODDARD, University of Wisconsin.

More than 60 calves have been dehorned with solutions containing antimony trichloride as the escharotic, salicylic acid as an analgesic, and flexible collodion as the carrier. Two solutions containing 28 and 38 per cent, respectively, of antimony trichloride, a commercial preparation containing an undisclosed amount of antimony trichloride, and stick caustic potash were compared. The 38 per cent solution, being more viscous, was easier to apply with a minimum of spreading. It also formed a firm film more rapidly, reducing the time required for application. The solution was applied by an eye-dropper with a spatula-shaped tip. The three solutions and caustic potash were about equally effective. The caustic potash was very irritating, whereas the calves dehorned with the antimony trichloride solutions showed no apparent pain during application. After about an hour, some exhibited a slight irritation, as shown by the shaking of their heads. The amounts required ranged from 1 to 2 g., with the smaller amounts used for the small horn buttons. The area covered should be kept to a minimum, covering only the button elevation. The amount necessary to remove the horn from some calves has been found to cause excessive swelling on others. The Guernsey calves seemed more resistant to escharotic action than did the Holstein, Ayrshire, Jersey and Brown Swiss calves.

The preparations were effective for calves between the ages of 3 and 10 days, but results were best at 3 to 5 days. Calves have been successfully dehorned at ages up to 17 days, but earlier treatment is recommended. The dehorning operation with the antimony trichloride solution took about twice as long as with caustic potash. Initial trials on dehorning kids have proved unsuccessful with all escharotics used.

P68 Comparison of Various Methods of Cooling Dairy Cows in Summer. D. M. SEATH, AND G. D. MILLER, Kentucky Agricultural Experiment Station and Louisiana Agricultural Experiment Station.

Three experiments were conducted in an effort to determine the efficiency of various methods of cooling cows in summer months. During the three trials the air temperatures as taken during daytime experimental periods averaged 88, 92.6 and 90.7° F., respectively. Cows either were placed in the sun for 2 hours prior to treatment or two lots of cows were used so that one could remain in the sun and serve as a check while the other received the cooling treatment. The effects of cooling treatments were measured by changes in body temperature and respiration rate.

Air movement as produced by a fan and directed at cows under a shade

shelter was more efficient in cooling than was the shade alone. The sprinkling of cows with water at 85° F. tended to cool them faster than did the fan, but at the end of 1 hour there was only a little difference. Sprinkling when combined with a fan was more efficient than either the sprinkling or fan alone.

A self-serving sprinkling device kept cows cooler than any of the other methods. This device was constructed by using a series of fog-producing self-cleaning spray nozzles attached to 0.5-inch pipe and suspended from the ceiling of a home-made bamboo shelter. Water under approximately 40 lb. pressure produced a mist-like spray which cows liked and used freely on warm sunny days. As a result, the body temperatures of the experimental cows remained normal or even lower and respiration rates near normal on relatively warm days.

P69 Relation of Management to the Let-down of Milk. C. E. KNOOP, Ohio Agricultural Experiment Station.

Twelve cows in the peak of lactation and two cows in the latter part of lactation were milked with a specially-designed milking machine in order to study the effects of temperature of udder wash water upon the let-down of milk. All factors relating to preparation and milking of the cows were standard, except temperatures of the udder wash water, which were as follows: cold (50 and 64° F.), warm (100° F.), and hot (132° F.). Related factors such as the inheritance of cows to milk at slow, medium, or rapid rates; conditions such as seasons of the year (spring 45° F. and summer 80° F.); effects of additional oxytocin; and variation in milking machine vacuum were included in this study.

Let-down of milk as determined by the amounts of milk taken from cows during the first 1 to 1.5 minutes of milking time and length of the total milking period were not influenced by temperature of the udder wash water. Even though further work is necessary, the results indicate that cows milk slowly or rapidly depending upon the size of the orifice ducts, strength of the sphincter muscles, and management routine.

P70 The Effect of Time of Milking after Milk Excretion on Total Milk Production. GERALD M. WARD AND VEARL R. SMITH, University of Wisconsin.

The effect of milking cows at different intervals after milk excretion was ascertained on five cows in various stages of lactation and levels of production. The mean production of each half of the udder was determined during a preliminary period by milking each half of the udder simultaneously into a separate container. During the preliminary period, the cows were prepared for milking by a hot water massage and the milkers

were attached 2 minutes later. During the experimental periods, the half of the udder which served as a control was milked 2 minutes after preparation and the other half was milked at 4, 8, 12, 16, or 20 minutes after preparation. One half of the udder alternately served as control and experimental. The experimental periods were 5 days in length, followed by a 2-day transition period. Treatments were at random. The experimental period was followed by a 5-day post-experimental period. Milk production for either half of the udder was significantly less when that half of the udder was milked at 12, 16 and 20 minutes after preparation as compared to the control period of milking 2 minutes after preparation. Milk production with the 4- and 8-minute treatments was not significantly different from the control.

P71 Silage or Winter Pasture for Dairy Cows. C. E. WYLIE, S. A. HINTON, AND L. R. NEEL, Tennessee Experiment Station.

In order to compare winter grazing with the feeding of silage in maintaining dairy cows in the winter months, feeding trials were set up. Three groups of Jersey cows were used. Group I was fed corn silage, hay and grain. Group II was fed hay and grain and allowed to graze annual winter pasture. Group III was fed hay and grain and allowed to graze rye and crimson clover pasture. In addition, this group was allowed to run on bluegrass and white clover pasture when the annual pasture field was considered too soft for tramping.

Trials were continued for four annual winter periods of 150 days each. Each year the production of the cows in Group III exceeded that of those in Groups I and II. Group II produced more than Group I in three of the four feeding periods, while Group I exceeded II one winter. Cows were able to run on annual winter pasture an average of 64 days per winter and on permanent pasture an additional 65 days. Days unsuitable for cows to be outside the barn averaged 21 per season, varying from a minimum of 9 to a maximum of 40.

P72 Sweet Sudan Grass as a Forage Crop for Dairy Cattle. K. A. KENDALL AND W. B. NEVENS, University of Illinois.

An 80 per cent sweet Sudan grass and 20 per cent soybean mixture containing 25-30 per cent dry matter when ensiled without added preservatives produced ensilage of excellent quality. It contained 1.42 per cent acidity. Twenty cows fed corn silage and sweet Sudan-soybean silage in a pair-fed, double reversal feeding trial produced 18,092 lb. fat-corrected milk and 17,480 lb. fat-corrected milk, respectively. The dry matter of the two silages as fed was 29.6 per cent for corn and 26.5 per cent for the sweet Sudan-soybean silage. For pasture, a sweet Sudan-soybean mixture

was compared with a common Sudan grass-soybean mixture. The former was more palatable to dairy cattle and remained greener throughout the pasture season. Sweet Sudan grass was found more resistant to disease than the common variety. The average prussic acid content of the sweet Sudan and common Sudan grass was 0.00204 per cent and 0.00216 per cent, respectively, when the crops were 18-30 inches in height.

P73 Pastures in Relation to Dairy Development in the South. R. H. LUSH, Tennessee Experiment Station.

The South has 25 per cent of the milk cows on 34 per cent of the farm land and produces only 17.8 per cent of the milk in the U. S. In 1944, over 40,000 less farms reported milk cows than in 1939, but the production per farm has increased about 1,000 lb. However, 80,932 more farmers, an increase of 47 per cent, now are selling whole milk off their farms. The dairy farms of the South are becoming fewer in number but larger in production than in the country as a whole. A continued shift in that direction calls for more uniform year-around production than where cream or home consumption was the chief outlet. Results obtained a few years ago at both the Middle and West Tennessee Experiment Stations favored nearly year-around grazing with little grain feeding. Recent information from the various Southern states has emphasized that where annual grazing crops are used, an early date of planting with liberal seeding and fertilizing of well-prepared land is essential for milk production. Improved practices involving the use of Sudan grass, millet, lespedeza and alfalfa-grass mixtures have helped even summer production. Old bluegrass pasture alone is not dependable but needs improving in two or more ways to be really productive.

P74 Irrigated Pastures for Dairy Cows. JOHN A. EWING, N. MADDEX, C. E. WYLIE, AND R. H. LUSH,¹ Middle Tennessee Experiment Station.

Results of irrigating permanent pastures to provide summer grazing are presented for 1945-1947. A bluegrass pasture of 13.2 acres was divided into nearly equal areas in 1945 and the same area irrigated by means of a sprinkling system, depending on moisture conditions. Two groups of Jersey cows, fed 2 lb. grain each daily and with access to alfalfa hay in racks, were used and numbers adjusted according to grazing available. Slightly more hay was eaten each year by the cows on non-irrigated pasture, but there was an average of 19 lb. greater gain in liveweight by cows on irrigated pasture. The average for three years, 1945-1947, shows cows on

¹ Engineers of the Tennessee Valley Authority. J. K. Underwood, Agronomist, and in the early tests, L. R. Neel, former Superintendent, cooperated.

irrigated pasture obtained 33 per cent or 47 more cow-days of grazing, produced 35 per cent or 1,193 lb. more milk, and returned \$39.85 or 27 per cent more above feed and irrigation costs per acre than did the cows on non-irrigated pasture. With normal depreciation costs of irrigation equipment included in the above figures, irrigation appears very profitable at present prices or where an adequate cheap water supply exists, and for maintaining summer milk production.

P75 Increasing the Production of Permanent Pastures through Renovation. J. B. SHEPHERD, R. E. WAGNER, R. E. HODGSON, W. J. SWEETMAN, AND C. G. MELIN, Bureau of Dairy Industry, and Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

At Beltsville, permanent pastures have shown an increase in grazing capacity of 16 per cent due to fertilization and a further increase of 10 per cent due to rotation grazing. Additional improvement has been made through renovation consisting of tillage and seeding to high yielding grasses and legumes. One Kentucky bluegrass pasture and one orchard grass-bluegrass pasture were renovated in the spring of 1945 and another of each in the spring of 1946. Renovated and check pastures were lined, manured, and fertilized alike. All pastures were rotation grazed. The year of renovation, a light first-cutting was harvested for silage and the pastures grazed afterwards. For 1945 to 1947, inclusive, the production of the pastures from grazing, in terms of standard grazing days (16 lb. T.D.N.) and good hay equivalent were, respectively: Kentucky bluegrass pasture check 177 days and 5,662 lb.; first year after renovation, 115 days and 3,892 lb. or 69 per cent; second year, 276 days and 8,828 lb. or 156 per cent; third year, 257 days and 8,214 lb. or 145 per cent. Orchard grass-bluegrass pasture check 174 days and 5,562 lb.; first year after renovation, 130 days and 4,159 lb. or 75 per cent; second year, 243 days and 7,788 lb. or 140 per cent; third year, 244 days and 7,810 lb. or 140 per cent. Averaging all years and all pastures, renovation increased grazing over fertilized, rotation-grazed permanent pastures by 21 per cent.

P76 Effect of Intermittent and Limited Winter Grazing of Rye Pasture on the Carotene and Vitamin A Content of Cows' Milk. R. G. WASHBURN AND C. F. MONROE, Ohio Agricultural Experiment Station.

Two groups of cows were fed a ration of corn silage, alfalfa hay and grain. Beginning in November, 1947, one of these groups was barn fed on this ration throughout the experiment, while the other group, in addition to this ration, was allowed to graze rye pasture for a brief period on

days when the weather was suitable. Due to the severity of the winter, the cows in the grazing group were not pastured as much as had been expected, but these cows ate well of the rye.

The carotene and vitamin A content of the milk in the preliminary period was 1,668 U.S.P. units vitamin A activity per l. for the control group and 1,778 units per l. for the pasture group. By November 25, cows in the control group had decreased to 1,382 units per l., while the group which had received 95 hours of pasture during this period had increased to 2,343 units per l. In the last week of January, 1948, the control group had further decreased to 1,009 units per l. and the other group which received only 17 hours on pasture had decreased to 1,176 units per l.

MANUFACTURING SECTION

M1. The Effect of the Addition of Ascorbic Acid to Milk on the Keeping Quality of Its Dried Product. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

Four different groups of samples of dried whole milk were prepared from milks containing differing amounts of added ascorbic acid. In each group the keeping quality of samples containing different concentrations of ascorbic acid was compared with a control which was made from the same milk but which contained no added ascorbic acid. In every case the control had the poorest keeping quality and the keeping quality of the other samples of the group increased as the amount of added ascorbic acid was increased. It was observed that the apparent ascorbic acid content (all substances which reduce 2-6 dichlorophenolindophenol) decreased rapidly at first but that later the apparent ascorbic acid increased. In samples of the best keeping quality the decrease was not great and eventually the concentration of apparent ascorbic acid became greater than the initial ascorbic acid concentration. From these observations it may be concluded that ascorbic acid protects the fat against oxidation. When sufficient ascorbic acid or reducing substances are present to protect the fat from oxidation until the dried milk generates reducing substances faster than they are destroyed in storage, prolonged keeping quality will result.

M2 The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

High heat treatment of milk reduces its redox potential (E_h) and improves the keeping quality of its dried product. The decrease in E_h depends on the conditions under which the milk is heated. Heating in an

open pan causes the least decrease in E_h . The same heat treatment in a full can, out of contact with air, which simulates that obtained with a tubular heater, causes approximately twice the decrease in E_h found by heating in an open pan. Heating deaerated milk for the same time and temperature in a full can causes approximately three times the decrease in E_h observed in open pan heating, indicating that there is a higher concentration of sulphhydryl groups, which are considered to be antioxidants.

Thirty samples of dried milk were prepared from normal and deaerated milk to determine the effect of the heat treatment of deaerated milk on the keeping quality of its dried product. The control sample of each pair was heated in a hotwell to the same temperature and for the same time as the deaerated sample. Twenty-seven of the pairs showed that deaeration before heat treatment improved the keeping quality of the dried product. These data indicate that heating deaerated milk preserves the substances which protect the fat more than the same heat treatment in contact with air.

M3 The Effect of Heat Treatment on the Reducing Systems of Milk.

S. T. COULTER, HERBERT HARLAND, AND ROBERT JENNESS, University of Minnesota.

Two methods have been used to determine the reducing capacity of fluid milk and dry whole milk—the thiamine disulfide method of Harland and Ashworth and the acid ferricyanide method of Chapman and McFarlane as modified in this laboratory. Based on work with simplified systems, the thiamine disulfide method measures essentially only those reducing groups produced as a result of heat treatment of the serum protein fraction of milk. The acid ferricyanide method includes these groups as well as ascorbic acid and reducing materials resulting from heat treatment of lactose in the presence of phosphate buffer or protein or both.

Data have been secured showing that the thiamine disulfide reducing substances reach a maximum during heat treatment of fluid milk and then decrease. The maximum reached is somewhat higher and the rate of decrease is slower in the absence of oxygen. A higher maximum is reached by high-temperature short-time heating (95° C. for 2 to 5 minutes) than by heating to a lower temperature for a longer time. The acid ferricyanide reducing groups, although showing some effect of oxidation during heating, continue to increase over long periods of heating.

The acid ferricyanide reducing capacity of dry whole milk may be increased as a result of heat treatment during drying. The thiamine disulfide reducing groups cannot be increased by the drying process. As a matter of fact, there is no change in the thiamine disulfide reducing capacity on heating systems which have a higher solids content than about 70 per cent. The maximum rate of production of acid ferricyanide reducing substances occurs on the heating of milk systems of about 90 per cent solids.

- M3-a The Heat Treatment of Milk Necessary to Prevent Lipolytic Action in Its Dried Product (A Preliminary Report).** GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

The heat treatment of milk used in making dried milk must be at least sufficient to prevent lipolytic activity. The time and temperature required to do this is not known. In certain samples of dried whole milk prepared from milk heated in a hotwell to high temperatures, the lipase still was active. Samples prepared from milks that had been heated for 30 minutes at 142, 152 or 162° F. (61.1, 66.7 or 72.2° C.), respectively, developed rancidity within 112, 126 and 140 days, respectively, when stored at 86° F. (30° C.). These data indicate that the lipase in milk is more resistant to heat treatment than commonly is supposed. Work is in progress on the destruction of lipase by short-time high-temperature heat treatment.

- M4 The Isolation of Compounds Responsible for the Stale Flavor Developed in Dried Whole Milk. I. The Distribution of Stale Flavor between the Fraction of Reconstituted Stale Whole Milk Powder.¹** R. McL. WHITNEY AND P. H. TRACY, University of Illinois.

This study was undertaken in the belief that, with the isolation and identification of the chemical compounds responsible for the stale flavor which develops in dried whole milk, a better knowledge of the mechanism of its formation could be obtained and preventive measures developed. Dried whole milk was prepared and stored under varied conditions to obtain a continuous supply of stale powder. Reconstituted stale dehydrated milk was separated into cream and skim milk. The cream was churned into butter and buttermilk, or washed until it "oiled-off." The butter and the washed cream were melted at 40° C., centrifuged, and filtered to yield butter oil and butter serum. The various fractions so obtained were reconstituted with appropriate fresh products to the composition of the original milk. These reconstituted milks were blended in concentration series with fresh whole milk and submitted to a judging panel to determine the threshold concentration of the stale fraction.

In all determinations made on the whole milk, cream, washed cream, butter and butter oil, the stale flavor component appeared to distribute itself according to the amount of fat present in the stale fraction. There-

¹ This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. 181 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

fore, it is indicated that the stale flavor component is concentrated in the butter oil.

M5 A Solubility Method for the Determination of Alpha and Beta Lactose in Dry Products of Milk. R. P. CHOI, C. W. TATTER, AND B. W. FAIRBANKS, American Dry Milk Institute, Inc., Chicago, Illinois.

Based upon the maximum rate of solution of lactose hydrate and upon the difference in initial solubilities of the *alpha* hydrate and the *beta* anhydride, a simple method has been developed for the determination of the two forms of lactose in dry products of milk. The essential steps consist of adding an excess of lactose hydrate to a known quantity of the sample, determining the solubility at several time intervals, and extrapolating to zero time to obtain the total initial solubility. The *beta* lactose content may be calculated easily from the fact that within a certain range of *beta* concentration the total initial solubility is the sum of the initial solubility of the *alpha* hydrate and of the quantity of *beta* lactose present. The *alpha* modification may be ascertained by determining the total lactose of the sample. Results for nonfat dry milk solids and dry whey solids by this method are in good agreement with those reported in the literature.

M6 The Viscosity and Heat Stability of Concentrated Milks Subjected to High Temperature Processing. B. H. WEBB AND C. F. HUFNAGEL, Bureau of Dairy Industry, U. S. Department of Agriculture.

The relative viscosity and heat stability attained by concentrated milks during their manufacture into various condensed or dried products is a factor in determining the amount of heat that can be applied and the physical characteristics of the finished product. Some of the relationships between the concentration, viscosity and heat stability of skim milks heated by different methods to temperatures between 160 and 280° F. have been studied. Skim milks of 23 to 28 per cent solids have sufficient heat stability to enable them to withstand a sterilization heat treatment after canning or before drying. At higher solids concentrations, coagulation occurs before the product can be sterilized. Heat treatments intermediate between pasteurization and sterilization were applied to skim milks of concentrations up to 45 per cent solids. Agitation during heating causes visible coagulation to occur sooner than when the milk is heated with a minimum of agitation. Continued agitation or pumping during the onset of coagulation drastically reduces the thickening that normally accompanies the formation of a visible coagulum.

M7 The Microbiological Keeping Quality of Bulk Condensed Milk. A. M. PEARSON, Ontario Agricultural College, AND F. E. NELSON, Iowa State College.

Samples of bulk condensed milk obtained from several plants at different times were held at 38, 44, 55, and, in some cases, 70° F. Changes in organoleptic characteristics, standard plate count and coliform count were followed at appropriate intervals, using a separate 2-oz. bottle of sample at each time interval. Titratable acidity, pH and pasteurization efficiency in a standardized ice cream mix were followed at the same intervals on four of the samples. Storage at 70° F. resulted in defect appearance within 2 days for nearly all samples. As the storage temperature was lowered, defects required considerably longer times to appear, only one sample becoming organoleptically unsatisfactory after 8 days at 38° F. Bacterial counts, acidity and pH became unsatisfactory somewhat before the samples were rejected for off-flavors. The lower the initial plate count or coliform count, usually the longer the samples remained satisfactory at any given storage temperature. When condensed milk from the same original lot was used, the count on the pasteurized mix in which it was incorporated largely was independent of the time and temperature, within the limits of this experiment, at which the condensed milk had been stored.

M8 The Use of Sweetened Condensed Whole Milk in the Manufacture of Caramels. J. J. SHEURING AND P. H. TRACY, Illinois Agricultural Experiment Station.

The quality and processing treatment of sweetened condensed whole milk should influence the color, body, texture and flavor of caramels into which it is manufactured, since the caramels consist of approximately 50 per cent of sweetened condensed whole milk. This study was undertaken to investigate the factors of forewarming temperatures and times, emulsifiers, composition, bacterial quality, salt balance and flavor of whole milk upon the sweetened condensed whole milk and the caramels into which it was manufactured. All the sweetened condensed whole milk was manufactured in the College creamery. Caramels were made in the kitchen of a candy company using commercial procedures.

Forewarming temperatures of at least 180° F. for 5 minutes for the milk were found to be desirable, as evidenced by light color, good flavor, shortness of texture, and firmness of body of the caramels. Seasonal variation of milk had no significant effect upon the flavor or body of the caramels. The addition of 0.075 per cent sodium citrate to the milk resulted in soft caramels, while the same amount of calcium oxide caused caramels to be too hard for cutting properly. The addition of 1½ ounces per 10 lb.

of fat sorbitan monostearate, and a mannitol derivative of beef fat improved the body of caramels, while an equal amount of soybean lecithin resulted in a sticky caramel that did not wrap efficiently.

M9 Influence of the Mineral Content of Water on the Properties of Ice Cream Mixes. ROBERT A. HIBBS AND W. A. KRIENKE, Florida Agricultural Experiment Station.

Several series of ice cream mixes, of average composition, were prepared from high fat content cream and plain condensed skim milk as the only sources of milk constituents, sucrose the only sugar, sodium alginate the stabilizer and water of varying degrees of hardness the diluent for completing the mixes. Distilled water was the diluent used in the control mixes. Processing procedures of the mixes were varied to include the homogenization of some complete mixes and of certain fractions of other mixes that consisted of the cream and part or all of the plain condensed skim milk with or without some of the water.

Effects of the minerals contributed by the hard water usually were reflected in increased clumping of the fat, retarded whipping of the mixes, reduced body and texture scores of the ice cream and increased curdy appearance of the melting ice cream. The most desirable mixes in this study had been homogenized as complete mixes.

M10 Observations on the Effects of Various Stabilizing and Emulsifying Materials on the Properties of Ice Cream. W. S. ARBUCKLE, R. B. REDFERN, AND L. F. BLANTON, North Carolina State College.

A comparison study has been made of common commercial ice cream mix stabilizers and emulsifiers. The study included stabilizing products, products containing a combination of stabilizing and emulsifying agents and stabilizing products plus emulsifying products. Data showing the effect of stabilizing and emulsifying materials on the properties of the mix and the finished ice cream have been secured.

Results indicate that some stabilizers affected the acidity and pH value of the mix. Viscosity measurements show that certain stabilizers produced an increase in the viscosity during the processing procedure, with no further change during an aging period. Some stabilizers caused an increase in viscosity of the mix during the aging period as well as during the processing procedure, and other stabilizers produced an increase in viscosity only during the aging period.

In comparison with stabilizers, the products containing a combination of stabilizing and emulsifying agents showed a more uniform acidity and pH value, somewhat lower surface tension value and greater viscosity of the mix, and a slower rate of melting of the ice cream. Emulsifying agents

had little effect on the acidity and pH value of the mix, produced a less viscous mix with a lower surface tension value, and caused a slower rate of melting in the finished ice cream.

M11 The Effect of a Mannitol of Beef Fat on the Whipping Qualities, Body and Texture of Ice Cream. RALPH NADEN, J. J. SHEURING, AND P. H. TRACY, University of Illinois.

A group of chemically prepared substances known to the trade as "emulsifiers" or "whipping agents" are being used in the ice cream industry. The use of mono- and di-glycerides of both plant and animal origin as emulsifying agents in ice cream has been patented. The inventors claim that these products impart faster whipping and improve the body and texture of ice cream.

The mannitol derivative of beef fat (MBF) used in this study was arbitrarily selected, as preliminary studies have shown that it had a satisfactory effect upon the physical properties of ice cream. The addition of MBF improved the whipping of mixes containing more than 6 per cent milk fat. MBF had practically no effect upon the viscosities of ice cream mixes. The use of 0.2 per cent MBF, when used in combination with commonly used stabilizers, improved the whipping qualities of ice cream mixes. Dehydration of mixes retarded the beneficial effects of MBF. Shrinkage of ice creams containing MBF exceeded control samples in all cases. MBF improved the whipping qualities of ice cream made from neutralized cream, butter or frozen cream. Aging of the mixes was found to be less necessary when MBF was used. MBF decreased the whipping time of unhomogenized mixes.

M12 A Study of the Fat Emulsion in Natural and Artificial Creams. WALTER E. SNYDER AND H. H. SOMMER, University of Wisconsin.

An extensive study of the fat emulsion in natural and artificial creams was made with particular emphasis on the phenomenon of "fat clustering" and the physical and chemical changes occurring during a slow warming and cooling treatment herein termed a "rebodying process". The chemical composition of the fat globule "membrane", the viscosity and whipping of cream, the reaction of creams to temperature treatments, the surface tension phenomena of milk and cream, and the response of milks and creams, natural and artificial, to additions and subtractions of various constituents all were utilized in this study.

Conclusive evidence is given to support the view that lecithin, agglutinin, colostrum, euglobulin of milk, and euglobulin of colostrum all enhance the rebodilying of cream. The composition of the membrane material of liquid globules differs from that of solid globules. Fat concentration,

lecithin concentration, salt composition, agglutinin, fat composition, lipolysis, average size and disparity in size of fat globules, and breed of cow were found to be important factors in the degree of rebodilyng response and whipping properties of natural and artificial creams. Marked surface tension changes were observed during the warming and cooling of milks and creams. Any correlation between agglutinin and surface tension values was not conclusively observed. Some evidence has been presented for the formation of a lipid-protein complex at the surface of artificially formed fat globules utilizing lecithin-butter oil-egg albumin and lecithin-butter oil-skim milk.

M13 Preservation of Milk for the Phosphatase Test. (GEORGE P. SANDERS AND OSCAR S. SAGER, Bureau of Dairy Industry, U. S. Department of Agriculture.

Experiments with preservatives showed that the following, arranged in increasing order of their destructive effects on milk phosphatase, will preserve fresh milk for 2 to 3 weeks at room temperature: 1.5 per cent of chloroform; 3.5 per cent of toluene; 2 per cent of borax; 0.1 per cent of formaldehyde; 0.05 per cent of mercuric chloride; and 0.15 per cent of hydrogen peroxide. The last three reduced the enzymic activity rapidly. Borax interfered in the test, yielding milk with a pH of about 9.7 instead of the optimal pH of 10. Chloroform was more effective than toluene as a preservative, although neither inhibited the enzyme appreciably. Other advantages of chloroform are that its presence can be detected easily by its odor, and it prevents clumping of the fat.

Phenolic compounds, such as cresols, naphthols and salicylates, should not be used because they react with BQC and produce a blue color. It is recommended that 1.5 to 2 per cent of chloroform be used to preserve fluid products for this test. For solid products, chloroform—e.g., 1.5 to 3 ml. in a 100-ml. container—can be put on a wad of cotton and placed with the sample in the container.

M14 Differentiation of Microbial Phosphatases from Milk Phosphatase.

RALPH P. TITTLER, OSCAR S. SAGER, AND GEORGE P. SANDERS, Bureau of Dairy Industry, U. S. Department of Agriculture.

Approximately 200 strains of microorganisms, representing 90 species and 23 genera, were tested for production of phosphatase in milk and other media. Positive tests at pH 10 were obtained only with *Aerobacter*, *Escherichia*, *Pseudomonas*, *Bacillus*, *Lactobacillus enzymothermophilus*, *Penicillium camemberti*, *Penicillium roqueforti*, *Aspergillus niger*, and *Aspergillus oryzae*. Many of these in milk gave negative tests. All strains of *Streptococcus*, *Leuconostoc*, *Lactobacillus* (except one), *Propionibacterium*,

Bacterium linens, *Alcaligenes faecalis*, *Oidium lactis* and yeasts gave negative tests at pH 10.

Microbial phosphatases in dairy products ("false positive" tests) can be distinguished from milk phosphatase by two methods: The former generally are not inactivated appreciably at 70° C. for 5 minutes, while the milk enzyme is destroyed completely at 70° C. for 1.5 minutes; the few microorganisms that produce an alkaline phosphatase, active at pH 10, generally produce also an acid phosphatase, active at pH 4-6, while milk phosphatase is not active below pH 8. The order of thermal resistance of phosphatases was: coliforms and *Pseudomonas* (> 90° C. for 5 minutes); spore-formers (> 70° to > 80° C.); *P. roqueforti* and *A. niger* (70° C.); and *P. camemberti* and *A. oryzae* (< 70° C.). The last two can be distinguished by their acid phosphatase.

M15 A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. J. H. HETRICK, Dean Milk Co., AND P. H. TRACY, University of Illinois.

A small tube heat exchanger built on Mallory principles was used to process the milk. A calculated time of 0.83 second was required to heat to holding temperature. After holding for the desired length of time, the samples were rapidly cooled in ice water. The Sanders and Sager test (J. Dairy Sci. 29: 507. 1946) was used to determine the phosphatase activity, and a value of 1 unit per ml. of milk was used as the standard for satisfactory inactivation of the enzyme.

A semi-logarithmic relationship between temperature and time over the temperature range of 143-185° F. was found to exist for the thermal destruction of phosphatase. This relationship can be expressed by the following formula: $T = 174 - 9 \log t$ where T is the temperature (° F.) and t is the holding time (seconds) required to inactivate the enzyme at temperature (T). From this formula another expression was developed which indicates the destructive effect of each second of holding at any temperature (T) in reducing the phosphatase activity to a phenol equivalent of 1 unit per ml. of milk. This expression is $D = \text{Antilog } 10 \frac{(T-174)}{9}$. The summation of the D values multiplied by the time in seconds at the corresponding temperature must be 1 or greater for any time-temperature cycle to satisfactorily inactivate the phosphatase. Data secured with various heating rates indicate that the mathematical solution is satisfactory for practical use in determining time-temperature relationships necessary to give a negative phosphatase test in milk with various heating methods. It follows that if phosphatase inactivation is used as the standard for adequate pasteurization, this mathematical method could be applied to determine proper time-temperature conditions.

M16 Isolation of Heat-induced Flavor Compounds from Milk. STUART PATTON AND DONALD V. JOSEPHSON, Ohio State University.

Heat-induced flavor compounds have been removed from skim milk, heated to 260° F. for 60 minutes, by direct extraction with redistilled ethyl ether. These substances may be concentrated by evaporation of the ether. The judgments of many impartial observers indicate that this concentrate has a strong "caramelized" odor, typical of heated milk. Fractionation of the flavor concentrate has been accomplished by selective solvent extraction and vacuum distillation. Solvent extraction has yielded three major fractions soluble in water, petroleum ether, and ether, respectively. To date only the first and third fractions have been subjected to further investigation. The ether soluble fraction contains the "caramelized" principle, whereas the water soluble fraction has more of a sulfide-type of "cooked" odor. Both of these fractions contain reduced sulfur in significant quantities. This evidence may explain why the sulfhydryl groups in heated milk disappear as the development of caramelized flavor progresses. Repeated tests for nitrogen on both of these fractions have been negative. Portions of the residue of the ether soluble fraction are vacuum distillable, but the principal "caramelized" substance, a yellow, sulfur-containing oil, could not be vacuum distilled under the conditions of the experiment.

M17 Some Observations on the Efficiency of High-temperature Short-time Pasteurization of Chocolate Milk. MARVIN L. SPECK, CHARLES D. COLVARD AND M. LEE SHUMAKER, North Carolina State College.

The heat treatment required for 99.99 per cent destruction of *Micrococcus freudenreichii* (no. MS66) in non-settling chocolate milk of different compositions and in whole milk was determined in laboratory pasteurization experiments using the technique previously described by Speck (J. Dairy Sci., 30: 975-981. 1947). The amounts of stabilizer (7.95-19.87 centipoise), sugar (5 and 8 per cent) and added nonfat dry milk solids (0 and 3 per cent) were varied in the chocolate milk studied. Differences in the per cent of these ingredients, as used in this study, had no appreciable effect on the heat treatment required to destroy the test culture. In comparing the resistance of *M. freudenreichii* in whole and in chocolate milk of the different compositions, no differences were observed at 165, 160, and 155° F. At 150, 145, and 143° F. longer exposures were required to destroy the test culture in chocolate milk than in whole milk. The adequacy of the standard for the holder method of pasteurization of chocolate milk, as contained in the Milk Ordinance and Code of the U. S. Public Health Service, 1939, therefore is questionable.

Preliminary experiments using a commercial HTST plate pasteurizer

have shown that temperatures of 161, 168, and 175° F. for 19 and 40 seconds gave reductions in the total bacterial count of chocolate milk comparable to that obtained by pasteurization at 145° F. for 30 minutes. The results have indicated that the final selection of a time and temperature for HTST pasteurization of chocolate milk may be as dependent upon the physical properties obtained on the product as on the minimum times and temperatures required to give satisfactory reduction in bacterial counts.

M18 Use of the Direct Microscopic Method for Pasteurized Dairy Products. M. J. PRUCHA AND VIRGINIA FRAZEE, University of Illinois.

The work was done in the Champaign-Urbana Public Health District laboratory during the last 2 years of the war. The community is operating under the United States Public Health Grade A milk ordinance. Samples of the pasteurized milk and cream were taken weekly from each milk plant and were examined by the standard plate method and also by the direct microscopic method. The purpose of the work was to determine whether the samples complied with the standard of the ordinance and not how closely the two methods agreed as to the exact count of bacteria in each sample.

In general, the two methods agreed on about 75 per cent of the samples as to whether the sample complied with the ordinance or not. Neither method alone gave complete information on the bacterial condition of the milk. In some cases, the milk was heavily loaded with bacteria but the plate method did not detect it. Sometimes the milk was contaminated subsequent to pasteurization and the direct microscopic method did not detect it.

M19 Bacteriophage Production by Cultures of *Streptococcus lactis*. F. J. BABEL, Purdue University.

Studies were conducted to determine the relationship between the number of *Streptococcus lactis* organisms used to inoculate given lots of milk and the bacteriophage titers of the milks, when the initial inoculations with bacteriophage were kept constant. As the amount of inoculum was decreased from 1.0 to 0.001 per cent, the final concentration of bacteriophage produced in the milks decreased. In these trials, secondary growth was most rapid in the cultures receiving the greatest inoculum and slowest in the culture receiving the smallest inoculum. A rather constant number of bacteriophage were produced from the bacterial cells added as the original inoculum when comparisons were made with the same culture and bacteriophage preparation. Two cultures of *S. lactis* which were sensitive to the same bacteriophage-free filtrate but which showed considerable difference in the time at which

secondary growth appeared were compared for bacteriophage production. One culture showed appreciable secondary growth in 6 hours, while the other culture was unusual because it was completely destroyed by bacteriophage and no secondary growth resulted. Both cultures produced approximately the same quantity of bacteriophage when the same number of organisms of each culture was added to milks inoculated with the same quantity of bacteria-free filtrate.

Several lots of milk inoculated with the same quantity of an *S. lactis* culture but with varying amounts of a bacteria-free filtrate active against the culture produced the same final bacteriophage titer. In these trials the initial bacteriophage titer could be varied from 10^1 to 10^7 and yet the final bacteriophage titer of the milks remained the same.

M20 Electron Microscope Studies of Bacteriophages Active against *Streptococcus lactis*. C. E. PARMELEE, P. H. CARR, AND F. E. NELSON, Iowa Agricultural Experiment Station.

Electron photomicrographs of bacteriophage particles active against *Streptococcus lactis* are presented. The photomicrographs of the cell-free bacteriophage particles were made from mounts on collodion membranes of a 1 to 100 water dilution of a cell-free whey filtrate having a titer of 10^{10} bacteriophage particles per milliliter. The photomicrographs of phage particles in the presence of bacterial cells were made from mounts prepared from mixtures of cell-free whey filtrates of bacteriophage and 24-hour cultures of susceptible *Streptococcus lactis* cells in 5 per cent whey solution. The mounts were dried for 12 to 14 hours, immersed in distilled water for 30 minutes, and again dried.

The mounts were prepared for observation and photography by shadowing with gold or platinum in a vacuum at an angle of about 75° . The bacteriophage particles are sperm-shaped and range from 0.18 to 0.28μ in total length. The spherical head of the sperm-shaped particle appears to be 0.06 to 0.09μ in diameter; the tail is 0.02 to 0.04μ wide and 0.12 to 0.19μ long.

M21 Some Factors Affecting the Rate of Acid Production by Cheese Cultures. H. C. OLSON AND FRANCIS D. COHENOUR, Oklahoma A. and M. College.

Various factors concerned with the daily propagation which might affect the rate of acid production by cheese cultures were studied. The factors which appeared to be of practical importance in increasing the rate of acid production by cheese cultures were increasing the solids-not-fat content of the milk, incubating at 70° F. rather than at 80 or 90° F., incubating until the cultures were thoroughly ripe, using enough inoculum to in-

sure a thoroughly ripe culture, and using freshly ripened cultures for inoculation of cheese milk.

The factors which appeared to have little influence on the rates of acid production by cheese cultures were: fat content of the milk used for daily propagation, bacterial content of the milk, temperature of heating of the milk above 165° F. for 30 minutes, and period of heating at 205° F.

M22 Methods of Controlling the pH of Fermenting Dairy Products and the Effects of pH Control. WAYNE I. TRETSVEN, Chicago, Illinois.

The hydrogen-ion concentration of dairy products is in part a function of chemical changes and directly affects the ensuing chemical changes as well as the physical qualities. In the manufacturing and handling of more or less concentrated, unsterile dairy products, such factors as temperature, light, and composition (concentration of moisture, sugar, salts, oxygen, etc.) have been the primary means of effecting the resulting changes.

The pH of cheese was changed by introducing desired gases. Cheese in carbon dioxide was found to ripen slightly slower than in nitrogen or *in vacuo* and much slower than when the pH had been increased by ammonia. A commercially produced fresh Cheddar cheese subjected to hydrogen chloride to reduce the pH to 4.2 failed to show any ripening in 15 months. The body and texture, color, and flavor were affected by the pH and could be controlled in part by pH.

Factors involved in changing the pH of cheese and other foods are size and shape, texture, moisture content, pH, time and concentration of reacting gas, variations in pressure, and temperature. By utilizing the procedures for altering and controlling the pH, new techniques in manufacturing and packaging have been found.

M23 Chemical Changes Occurring in Limburger Cheese during Accelerated Ripening. W. K. STONE AND S. L. TUCKEY, University of Illinois.

M24 A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-frequency Heat. F. V. KOSIKOWSKY, B. L. HERRINGTON, AND A. C. DAHLBERG, Cornell University.

A number of batches of raw milk were made into American Cheddar cheese, which then was pressed overnight in Wilson square hoops. The pH of these cheeses varied from 5.1 to 5.3. The cheeses then were cut up into blocks (1.5 × 4 × 5.25"), packaged in Parakote, and heated directly by placing between the two electrodes of an experimental R.C.A. radio-frequency oscillator. The oscillator had a possible power output of 750 watts at 150

megacycles. The time required for heating 1.3-lb. packages of cheese to the desired temperature ranged from 1.5 to 2.7 minutes. Temperatures to which cheese were heated ranged from 117 to 155° F. After attaining the desired temperature, the cheeses were placed in cardboard boxes, air cooled, or held for 15 seconds and then air cooled at 70° F.

Raw milk cheeses held for 2 days at 50° F. and heated to as high as 146° F. retained their physical form. There was no oiling-off, and after cooling no noticeable difference was apparent between the heated and unheated samples. As the raw milk cheese became older, lower heating temperatures were required to obtain a desirable physical form.

Heating of most 2-day-old cheeses to temperatures as low as 130° F. with radio-frequency heat followed by air cooling at 70° F. destroyed on the order of 99.9 per cent of the bacteria and produced a phosphatase-negative cheese.

After ripening at 60° F. for 2 months, cheeses heated by radio-frequency showed definite signs of curing, in some cases closely approaching that of the raw control samples. Curing was indicated by increases in soluble protein, fatty acids, bacteria, and by activity of the decarboxylase enzyme system as determined by tyramine increases and finally by body breakdown and increase in flavor intensity. Some very high scoring cheeses were produced using radio-frequency heating, although extensive overheating produced oily flavors in some cheeses, while in others the cheese did not cure fully.

M25 Increasing Efficiency and Reducing Costs in the Manufacture of Cheddar Cheese. D. M. IRVINE AND W. V. PRICE, University of Wisconsin.

Economic adjustments of Cheddar cheese factories in Wisconsin are causing major changes in plant operations. The number of factories is decreasing and production per factory is increasing. This trend suggests the possibility of greater utilization of labor-saving techniques and devices.

Practical evidence indicates that the stirred-curd method of manufacturing Cheddar cheese may be more readily mechanized than the accepted matted-curd process of manufacture, but the characteristic openness of the stirred-curd type does not satisfy Wisconsin State Brand standards. Experimental attempts to correct this fault now are in progress. Tentative conclusions indicate that composition and flavor of normal stirred-curd Cheddar duplicate the conventional procedure. Texture is improved by maintaining temperatures approximating 100° F. during the entire draining process. Rapid cooling of the curd during this period promotes openness, inferior flavor, higher moisture, and slightly more acidity. Mechanical devices are being tried with some success to eliminate hand labor of the making process.

M26 The Use of Nonfat Dry Milk Solids in the Manufacture of Cheddar Cheese from High Fat Content Milk. G. H. WILSTER, C. E. JOHNSON, AND P. R. ELLIKER, Oregon State College.

Results on 54 batches of experimental cheese indicate that Cheddar cheese of high quality can be manufactured from high fat content pasteurized milk adjusted with reconstituted nonfat dry milk solids to a fat to solids-not-fat ratio of 1: 2.35. A high preheat type of powder was used. Preliminary studies also were carried out under commercial conditions. Some of the batches of cheese were made with dry milk manufactured by a low heat treatment method.

Body and texture defects, such as crumbly and brittle, occurred when milk containing more than 4.7 per cent fat was standardized with reconstituted nonfat dry milk solids to a ratio of fat to solids-not-fat of 1: 2.35. The addition of 0.025 per cent calcium chloride to the cheese milk, and holding the reconstituted nonfat dry milk solids for a period of time before addition to the milk were found to have no advantage. Excessive agitation during cooking was found to be conducive to a crumbly curd during cheddaring and to a low moisture cheese. After the whey was drained, the curd was allowed to mat for 15 to 20 minutes before the first turn was made. Further studies are being made to determine the type of nonfat dry milk that is most suitable for use in standardizing milk for Cheddar cheese.

M27 The Problem of Sampling Cheddar Cheese for Analysis. WILLIAM C. WINDER AND WALTER V. PRICE, University of Wisconsin.

Moisture in Cheddar cheese is not distributed uniformly and Cheddars from the same vat lot are not alike. The usual sample removed by trier from a Cheddar overestimates the moisture content of the edible portion of the cheese. In this study, commercial lots of Cheddar cheese were sampled with a long trier. Three plugs were taken from both flat surfaces of each Cheddar in the manner suggested in "Methods of Analysis", 6th Ed., AOAC. Each plug was analyzed; the average percentage of moisture in all plugs for a Cheddar was called the "moisture content" of that Cheddar.

The results show that, no matter how it is taken, the plug sample must be regarded as merely an estimate of the moisture content of the vat lot of cheese. The minimum sample for reasonable estimates consists of plugs which remove the vertical axis of the Cheddar, excluding only the rind portion necessary to seal the openings in the cheese. According to the data, such a sample, taken from one Cheddar of a normal vat lot, estimates the moisture content of the lot within control limits of 0.61 per cent. When two, three or five Cheddars of the vat lot are sampled in this same manner, the moisture content can be estimated within control limits of 0.44, 0.36 and 0.27 per cent, respectively.

M28 The Influence of *Oospora lactis* in Promoting Changes in the constants of Cheese Fat during the Ripening of Cheddar Cheese.

S. L. TUCKEY, W. O. NELSON, AND R. V. HUSSONG, University of Illinois.

Oospora lactis when grown with *Streptococcus lactis* in cream produces marked lipolysis of the fat. To determine its effect on the fat of Cheddar cheese during ripening, three lots of milk were made into six batches of cheese. One half of each lot of milk was made into cheese containing the *O. lactis*, while the other half served as a control batch. Three different concentrations of inoculum of *O. lactis* were used as follows: 0.05, 0.18 and 1 per cent. The inoculum was a 3-day-old culture of *O. lactis* and *S. lactis* in sterilized 35 per cent cream, which contained at time of use 4,900,000, 2,700,000, and 4,300,000 *O. lactis* per ml., respectively, for the three lots. The fresh cheese curd showed 4,000, 14,000, and 420,000 *O. lactis* per g., respectively.

The following fat constants were determined at monthly intervals: acid number (Breazeale and Bird), Reichert-Meissl, Polenske and saponification numbers. The fat of the three lots of inoculum showed marked rancidity as determined by taste and titration. However, in the lots of cheese containing the *O. lactis*, the acid number was the only fat constant showing any consistent change from the control lot which would indicate any difference in the hydrolysis of the fat between the lots. The batch containing 0.18 per cent inoculum had the least desirable flavor of the different lots of cheese. The inoculum at time of use had an "old cream" and "fermented" flavor. The "fermented" flavor persisted during four months of ripening. No rancid flavors were noted in any of the lots of cheese.

M29 Studies of Sources of the Typical Flavor in Cheddar Cheese.

HAROLD E. CALBERT AND WALTER V. PRICE, University of Wisconsin.

Twenty-eight lots of Cheddar cheese and eight lots of cheese of various other varieties were analyzed for diacetyl. A modified colorimetric method was used. Although diacetyl was found in all samples, 75 per cent contained less than 1 p.p.m.

The Cheddar cheese was divided into two groups depending on its flavor. Group I included all lots with no flavor defects; 78 per cent of this group contained less than 0.5 p.p.m. of diacetyl. Group II included lots with adverse flavor criticisms. The diacetyl content of the latter group varied from 0.2 to 3.35 p.p.m. A small quantity of diacetyl seems to be essential in the typical flavor and aroma of Cheddar cheese. These studies are being continued to determine the importance of diacetyl in typical cheese flavor.

M30 Influence of Temperature of Ripening on the Concentration of Tyramine in American Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, Cornell University.

Two batches of pasteurized milk of excellent sanitary quality each were divided into three cheese vats and made into American Cheddar cheese by the usual procedure. Two per cent of Hansen's lactic starter was added to one vat of milk, 2 per cent of DK cheese starter (containing a special strain of *Streptococcus faecalis*) was added to another vat of milk, and 1 per cent of each of the starters was added to the third vat of milk. The cheese from each vat of milk was cured for 1 year at 40, 50 and 60° F.

The cheese was analyzed for chemical composition and bacterial content and was scored at regular intervals. In agreement with previous research from these laboratories, it was found that the least tyramine developed in the cheese containing the usual lactic starter, and the most developed in cheese made with both commercial lactic and DK cheese starters. Furthermore, the intensity of Cheddar flavor increased with increased tyramine content. The amount of tyramine in cheese increased as the temperature of curing increased and so did flavor development. Cheese made with commercial lactic starter cured for 1 year at 40° F. contained only 3 γ of tyramine per g. of cheese; at 60° F., it contained 37 γ per g. The highest tyramine contents were found in 1-year-old cheese made with both commercial lactic and DK cheese starters. This cheese cured at 50° F. contained 1,369 γ per g., and at 60° F. it contained 2,554 γ per g. cheese.

M31 Methods for Studying the Ripening of Cheese. H. H. SOMMER AND W. J. HARPER, University of Wisconsin.

The method of Van Slyke and Hart (1902) was modified by using a Waring-type blender in place of grinding with sand, to suspend the cheese in water, and by making the first separation by acidification to pH 4.7.

In the original Van Slyke and Hart procedure, the first separation was into water extract of the cheese at 50° C. and water insoluble residue. From the water extract was obtained a precipitate on acidification which they called "paranuclein". From the water insoluble residue a 5 per cent NaCl-soluble fraction which was called "unsaturated paracasein lactate" was obtained.

In view of the later findings of Van Slyke and Bosworth (1912) on the water and 5 per cent NaCl solubility characteristics of paracasein as influenced by reaction and calcium salts, the significance of Van Slyke and Hart's "unsaturated paracasein lactate" and "paranuclein" is doubtful. Various suggestions have been made that the water extraction of the cheese should be made with control of such factors at pH, NaCl concentration, and CaCl₂ concentration. However, since the fraction here involved is

essentially unhydrolyzed paracasein, it has little or no significance in studies of proteolysis in cheese ripening. Accordingly, in the present method, the first separation is the precipitation of materials that are insoluble at pH 4.7 (50° C.), and subsequent separations are applied to the filtrate using the general scheme of Van Slyke and Hart.

M32 The Effect of Added Amino Acids on Flavor Development in Cheddar Cheese. R. J. BAKER AND F. E. NELSON, Iowa State College.

The possibility that the addition of various amino acids to cheese curd made from pasteurized milk might furnish the substratum for production of the typical flavor and aroma compounds of a fine raw milk cheese was the basis of this study. Of the amino acids normally present in casein, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, and valine were used. They were added with the last increment of salt, at the rate of 0.5 g. (0.04 per cent) to a 2.5-lb. quantity of curd, which then was pressed in special small hoops, giving a cheese 5 inches in diameter and about 2.5 inches high.

On the basis of organoleptic examinations every 4 weeks during a 24-week curing period for two complete series of cheese, histidine produced a definitely inferior cheese, having a pronounced unnatural flavor due to the amino acid itself. Glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid and valine showed a possible tendency to increase the desirable flavor level in the test cheese. These eight amino acids were used in 0.5 g. (0.04 per cent) and 1.5 g. (0.13 per cent) amounts in a third series of cheese. Plate counts and direct microscopic counts also were made. No consistent and significant differences were established in this series.

Separate additions of 19 amino acids to the curd of Cheddar cheese made from pasteurized milk had no consistent and reproducible effects upon flavor development or bacterial growth.

M33 Studies of Amino Acids in Cheddar Cheese during Ripening. W. J. HARPER AND A. M. SWANSON, University of Wisconsin.

An investigation was made to determine some of the individual compounds formed by protein degradation during the ripening of Cheddar cheese. Analyses for nine amino acids were made by microbiological assay on hydrolyzates of cheese (I), water extract of cheese (II), and the water extract minus heat coagulable material (III). Determinations also were made for the "apparent free" amino acids in the unhydrolyzed water extract (IV). These amino acids were aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline and valine. Five repre-

sentative cheese, varying in age and degree of cheese flavor, were studied.

All of the amino acids were found to be present in every fraction (I, II, III, and IV) of each cheese. The quantity of all amino acids in II, III, and IV increased directly with age. Regardless of this increase, a direct relationship was found between the degree of cheese flavor and the majority of the different amino acids present in fractions III and IV. Further investigation is being undertaken to determine whether amino acids contribute to cheese flavor or are merely indicative of ripening changes.

M34 The Influence of Various Lactobacilli and Certain Streptococci on the Chemical Changes, Flavor Development and Quality of Cheddar Cheese. R. P. TITSLER, G. P. SANDERS, H. R. LOCHRY, AND O. S. SAGER, Bureau of Dairy Industry, U. S. Department of Agriculture.

Cheddar cheese was made from milk of good quality which was pasteurized and divided equally into two lots. Lactic starter was used in one lot and lactic starter plus a supplemental starter was used in the other lot, in 110 experiments. Bacterial (quantitative and qualitative), chemical (nitrogen fractions, titratable acidity, pH, lactose, biacetyl, moisture), and physical (plasticity, elasticity, tensile strength) determinations were made at regular intervals.

Streptococcus faecalis, *Leuconostoc citrovorum*, *Leuc. dertranicum*, and *Leuc. mesenteroides* had little or no effect on proteolysis or quality of the cheese. *S. liquefaciens* greatly increased proteolysis and produced an objectionable flavor. *Lactobacillus casei*, *L. plantarum*, *L. brevis*, and *L. fermenti* grew rapidly in the cheese. *L. bulgaricus*, *L. helveticus*, *L. lactis*, and *L. acidophilus* were not detected after 2 weeks and had little or no effect on proteolysis or quality. *L. casei* increased the acidity and the rate of softening of the body but did not increase soluble nitrogen; it increased the development of flavor but later caused an acid flavor and "short" body. *L. fermenti* produced gas and a very objectionable flavor. *L. brevis* produced an objectionable flavor. Some strains of *L. plantarum* increased development of desirable flavor without producing excessive acidity and ultimately may prove useful for faster curing.

M35 Some Physiological Effects of Dietary Lactose.¹ JESSIE ELIZABETH FISCHER AND T. S. SUTTON, The Ohio State University.

The rate of passage of food residues through the digestive tract of rats was estimated by fecal recovery of a dye (Sudan III) which was administered by stomach tube. Food residues passed through the digestive tract more rapidly when lactose was administered by stomach tube than when sucrose was similarly given. A similar effect of lactose incorporated in

¹ Research supported by The American Dry Milk Institute, Inc.

the ration depended upon the percentage of lactose included. The order of rapidity of food residue passage produced by the different rations tried was: 30 per cent > 20 per cent lactose > 40 per cent lactose > basal corn-starch. The severity of diarrhea observed was greatest in the animals on the 40 per cent lactose ration despite the foregoing results. A 40 per cent lactose ration fed to young rats increased the amount of both water and dry matter in the contents of the digestive tract. Despite persistent watery diarrhea in the lactose-fed group, dehydration did not occur, although water intake was not increased.

Roentgenologic studies of the passage of a ration containing 60 per cent lactose indicated that the chief site of stasis was the cecum, although the stomach seemed to empty somewhat more slowly. Activity of the longitudinal muscle of isolated small intestinal segments failed to indicate that lactose exerts a direct stimulatory effect on this muscle.

M36 The Limitations of the Refractometer Readings of Milk Serums in Detecting Watered Milk. L. R. ARRINGTON AND E. L. FOUTS, University of Florida.

Refractometer readings were made of the serum of mixed Holstein and mixed Jersey milk and on the same samples after 3, 5, 7, and 9 per cent water had been added. Readings were made on the acetic serum, copper serum and sour serum as directed by the Association of Official Agricultural Chemists. Also, a modification of the sour serum method was made by adding 1 per cent culture and incubating the milk at 70° F. for 15 hours. The freezing points using the Hortvet cryoscope were determined on the original and diluted samples. The percentages of fat, total solids (Mojonnier) lactose and chlorides were determined on the original samples.

On the basis of standards listed in the official methods of the Association of Official Agricultural Chemists, added water in amounts up to 9 per cent in milk of normal fat and total solids content was not positively detected by any of the serum methods. The addition of smaller amounts of water to milk with low fat and total solids could be detected. The standards given for each of the official methods appear to be too high for normal milk. Sour serums prepared by the official method gave inconsistent values. When the method was modified by addition of 1 per cent culture followed by incubation for 15 hours at 70° F. the values were more consistent and averaged 1 refractometer degree lower than serums from natural souring.

M37 The Application of Flame Photometry to Determinations of Calcium, Potassium and Sodium in Milk. W. A. KRIENKE AND NATHAN GAMMON, JR., Florida Agricultural Experiment Station.

Two flame photometers were used in this study, one a combination of a Lundegardh burner with a Beckman quartz spectrophotometer and the

other a Perkin-Elmer model 52 A spectrophotometer. A comparison of results is given.

The procedure for analysis of milk for sodium and for potassium is to ash the sample, take up the ash in hydrochloric acid, evaporate to dryness, take up the ash in a known volume of 0.01 N hydrochloric acid, fiber, and atomize in the flame. The application of the internal standard technique involves the addition of a known quantity of lithium chloride before making to final volume.

Calcium presents a more difficult problem because of the depressing effect of phosphate and sulfate ions on the excitation in the flame. Two procedures, one a spectroscopic buffer, and the other a lead precipitation, are suggested for control of phosphate and sulfate ion variations. The importance of using standards containing the same ions and in approximately the same relative concentrations as present in the prepared samples is emphasized.

M38 A Rapid Method for the Determination of Nitrogen in Milk Products by Direct Nesslerization of the Digested Sample. J. H. HETRICK, Dean Milk Company, Rockford, Illinois, AND R. McL. WHITNEY, University of Illinois.

Interest in the changes in the protein fractions of milk made it necessary to develop a more rapid method for the determination of nitrogen than was currently in use. As developed, the test consists of: (a) digestion in a micro-Kjeldahl apparatus of a diluted sample with potassium sulfate and sulfuric acid until the sample clears, (b) completing the digestion with 30 per cent hydrogen peroxide, (c) diluting the digested sample using a gum acacia solution, (d) addition of a Nessler's reagent to an aliquot of this solution, (e) measuring the per cent transmission spectrophotometrically at 420 $m\mu$, using a potassium dichromate solution as a standard blank, and (f) computing the nitrogen content from a standard concentration curve.

The effects of the following variables upon the determination were investigated: concentration of potassium sulfate, sulfuric acid, hydrogen peroxide, gum acacia, type and amount of Nessler's reagent, time or color development, and temperature of color development. Results of analyses using the technic indicate satisfactory reproducibility with a standard deviation of 1.5 per cent of the nitrogen content of the sample. When compared to the A.O.A.C. procedure, the results were found to average approximately 2 per cent below those of the official method. The saving in time, while dependent upon the apparatus available, is considerable.

M39 Studies on Separation and Fractionation of Casein. E. C. HAGBERG AND A. M. SWANSON, University of Wisconsin.

Studies have been made on the precipitation of casein and its frac-

tiation into *alpha* and *beta* casein by use of Warner's method. This method is based on the fact that at 2° C., *beta* casein has a higher isoelectric point than *alpha* casein. Fractional precipitation is accomplished by approaching the isoelectric point from the acid side.

The dried samples were prepared by lyophilizing. This resulted in material which was more readily soluble than that dried by alcohol and ether methods. Light transmission observations were made on the precipitation of casein and its fractions. The degree of dispersion as evidenced by turbidity varies with the pH, and the changes leading to acid precipitation were gradual. The relationship between turbidity and pH was different for the two fractions of casein. *Beta* casein was found to have a higher relative viscosity in the pH range of 5.5 to 9.0.

Analytical results showed that *alpha* casein contained a higher percentage of total phosphorus, aspartic acid, glutamic acid and tyrosine. Significant differences were found in rate of hydrolysis in alkali. Alpha casein was most readily hydrolyzed.

M40 The Fractionation of Milk Fat by Molecular Distillation. E. L. JACK AND MRS. L. B. OLSEN, University of California.

Milk fat has been subjected to molecular distillation using a cyclic still. The fat was passed over the distilling surface twice at each fractionating temperature. Attempts to distill exhaustively at each temperature were too slow to be practical. The first fraction was removed at 140° C. and the temperature was increased 10° C. for each succeeding fraction until substantially all the fat had been distilled. The last distillate was removed at 190° C., making seven fractions including the residue.

Analyses of the fractions obtained from a fat having a saponification number of 236 and an iodine number of 28.5 showed that the saponification number of the fractions decreased from 263 to 205 progressively with succeeding fractions, while the iodine number increased progressively from 16 to 43. The non-saponifiable components were segregated among the fractions. Fractionation by molecular distillation yields fractions of different chemical properties from those secured by solvent precipitation.

M41 The Measurement of Free Fatty Acids in Dairy Products. H. A. HOLLENDER, S. R. RAO, AND H. H. SOMMER, University of Wisconsin.

A method has been worked out for the estimation of the free fatty acids during lipolytic studies on fat in the form of emulsions. This method can be applied to the estimation of free fatty acids in powdered milk, cream and other high-fat dairy products. The proposed method utilizes the high solubility of free fatty acids in 95 per cent alcohol. Five to 10 g. of the

substrate (powdered milk or cream) is placed in a centrifuge tube of about 100 ml. capacity. Fifty milliliters of neutral 95 per cent ethyl alcohol are added, maintaining 85 per cent concentration of alcohol in the mixture. The mixture then is boiled for 30 seconds, cooled and centrifuged until clear. The supernatant is decanted off and the extraction repeated with fresh alcohol. The combined supernatants are titrated with 0.02 N alcoholic alkali, using a micro burette and 5 drops of 1 per cent phenolphthalein as the indicator.

Recovery studies on fatty acids added to milk and cream have resulted in quantitative recoveries of oleic, palmitic and butyric acids and mixtures of these. Further studies on the recovery of other fatty acids are being carried out, along with work to determine the amount of non-fatty acid acidity that will be extracted by the alcohol.

M42 The Determination of Linoleic Acid in Butterfat. P. S. SCHAFER AND GEORGE E. HOLM, Bureau of Dairy Industry, U. S. Department of Agriculture.

Samples of the fat acids of butter oil, from milks produced during the spring, summer, and winter months, were isomerized with alkali and their absorptions in the ultraviolet region of the spectrum, 224–274 $m\mu$, determined. The linoleic acid content was calculated from the degrees of absorption at 234 and 268 $m\mu$ and the specific absorption coefficient of diene and triene conjugations at 234 $m\mu$, and 234 and 268 $m\mu$, respectively. In fat acid samples containing 1 and 3 per cent of added linoleic acid, the maximum deviations from theoretical recovery were 0.008 and 0.015 per cent, respectively. The linoleic acid content of the milk fats ranged from 2.11 to 2.40 per cent, the latter being the value obtained on milk fat produced during the summer months.

M43 Retention of Ascorbic Acid, Changes in Oxidation-reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. R. W. BELL, Bureau of Dairy Industry, U. S. Department of Agriculture.

The tendency of milk to develop an oxidized flavor is a limiting factor in the preservation of milk by freezing. Oxidation-reduction-potential changes and decreases in ascorbic acid during the onset of the off-flavor in frozen milk were measured by examining the thawed product. A strong oxidized flavor developed in frozen milk with but slight decrease in ascorbic acid content, and the rate of development was much slower in milk that had been fortified with the acid. Deaeration aided in the preservation of ascorbic acid but only slightly retarded the onset of the off-flavor.

It was concluded that a low oxidation-reduction potential obtained by

adding ascorbic acid to fresh milk greatly retards but does not prevent the development of an oxidized flavor in frozen milk. However, it does not increase the retention of vitamin C in the form of ascorbic acid.

M44 The Effects of the Treatment of Milk and the Subsequent Storage of Cream and Butter below Freezing Temperatures upon the Sensitivity of Fat to Oxidation as Determined by the Re-emulsification Test. VLADIMIR N. KRUKOVSKY, E. S. GUTHRIE, AND F. WHITING, Cornell University.

The milk fat (triglycerides) is relatively stable in fresh milk and is not involved in the reaction which produces the oxidized flavors. However, the fat undergoes oxidation in the presence of ascorbic acid, resulting in the development of the objectionable flavors and losses in vitamins E, A and carotene. The susceptibility of milk fat to this type of deterioration is determined primarily by the treatment of milk and cream, the type of product held (butter, cream, fat), and the conditions of storage.

Cream separated from milk depleted of its total vitamin C content by oxygenation during the pasteurization was found free of all flavors at the end of the twelfth month of storage at 0 to 3° F. However, the storage life of fat, as determined by the re-emulsification test, was terminated at the end of 4 to 6 months, depending upon the conditions of processing. Only fat from butter churned from cream pasteurized at 160 and at 170° F. and pure fat retained their abilities to resist this type of deterioration at the end of 2 years of storage at 0-3° F.

While the oxygenation of milk results in the prevention of oxidized flavors, the latter can be induced again by the addition of ascorbic acid.

M45 Ascorbic Acid Oxidation in Milk by Preformed Hydrogen Peroxide VLADIMIR N. KRUKOVSKY, Cornell University.

A study was made to ascertain if the inactivation of peroxidase in milk, as produced by heat (Zilva, at 76.7° C.), would result in non-reactivity of ascorbic acid and H_2O_2 and if the ability of milk to promote ascorbic acid oxidation by added H_2O_2 can be restored either by the addition of horse radish peroxidase, prepared according to Sumner and Gjessing, or of Cu.

While ascorbic acid was oxidized rapidly and completely by H_2O_2 added to milk pasteurized at 61.1° C. for 30 minutes, H_2O_2 was not utilized for the oxidation of ascorbic acid, neither in the milk pasteurized at 76.7° C. nor in milk to which H_2O_2 was added in excess of the amount needed to complete ascorbic acid oxidation prior to pasteurization of milk at 61.1° C. This fact was established by the readdition of both reagents to milk after the heat treatments. However, the foregoing reaction was induced again by the addition of peroxidase to non-reactive milk. Only a part of

ascorbic acid was oxidized rapidly by H_2O_2 with Cu as a catalyst in non-reactive milk.

These results indicate that peroxidase in milk may play an important part in the reaction involving ascorbic acid oxidation in the presence of added H_2O_2 .

M46 Stimulation of the Oxidized Flavor in Homogenized Milk by Daylight as Governed by the Vitamin C Content of the Milk. E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY, Cornell University.

A study was made of the susceptibility of homogenized milk to the development of the oxidized flavors when it was exposed to light in the presence or absence of vitamin C in milk. For this purpose some of the milk was first depleted of its total vitamin C content either photochemically or by hydrogen peroxide, and then pasteurized at 143° F. for 30 minutes.

Irrespective of the pressure, the quick partial photochemical oxidation of ascorbic acid resulted in the promotion of the oxidized flavors in the homogenized milk, whereas its complete oxidation resulted in the prevention of the oxidized flavors. The oxidized flavors were not induced by the addition of ascorbic acid to homogenized milk which was first depleted of its total vitamin C content by exposure to light and subsequent pasteurization prior to homogenization, providing the pressure used was above 1,000 lb. per square inch.

M47 A Study of Seepage from Bottles of Homogenized Milk. E. O. HERREID, J. FRANCIS, AND P. H. TRACY, Illinois Agricultural Experiment Station.

Milk sometimes appears in small amounts around the edge of the fiber disc cap and around the wire staple which anchors the paper tab on the bottle cap. Milk also sometimes appears on the lower edge of the metal cap which covers the mouth and lip of the bottle. In the market milk industry this condition has been referred to as seepage, leakage, creeping, crawling and swelling.

Fiber disc caps and bottle cap seats were measured and slight variations in their size and shape were observed, but even the most extreme variations could not be correlated with seepage. However, the fiber disc and metal caps varied in their ability to hold a tight seal, a factor which did affect seepage.

The principal cause of seepage is the expansion of gases and vapor in the headspace beneath the cap when the bottles are exposed to elevated temperatures. When seepage occurs, the cap usually is firmly set and the pressure reaches a certain point and is suddenly released, forcing out a small quantity of milk. Seepage usually does not occur when the pressure

is released slowly during exposure of the bottles to elevated temperatures. These changes in pressure were observed with a manometer attached to the bottles. The incidence of seepage is increased by the excessive incorporation of air in milk during processing operations and exposing it to elevated temperatures during hauling and delivery.

M48 The Leucocyte Count of the Complete Milking of Normal Animals for Complete Lactation Periods. E. O. ANDERSON, University of Connecticut.

The average arithmetic cell count of 19,710 samples from the complete milking of 18 mastitis-free cows over their complete lactations was found to be 160,000 per ml. as compared to a cell count of 380,000 and 220,000, respectively, for cows chronically infected with staphylococci and streptococci other than *Streptococcus agalactiae*. The average cell count of composite samples of the herd milk taken over the same period was 380,000 per ml.

The average arithmetic cell count of mixed herd milk obtained at the receiving deck of milk plants from 20 herds infected with *S. agalactiae* was found to be 880,000 per ml. as compared with a cell count of 660,000 per ml. in mixed milk from 11 herds free of *S. agalactiae*. From the data available, a parabolic curve fitted by the method of least squares indicates that when the percentage of *S. agalactiae* cows in a herd reaches 36 per cent or over the average cell count of mixed herd milk will probably be a million cells per ml. or more.

M49 Effect of Some Water Constituents on a Quaternary Salt. W. S. MUELLER AND D. B. SEELEY, University of Massachusetts.

This study was undertaken to obtain more information on the controversial problem of whether the constituents of water, especially those of hard water, affect the germicidal potency of quaternaries. Natural waters and also waters to which various common ions had been added were used to prepare a 200 p.p.m. quaternary solution, which was tested against *Escherichia coli*.

No close correlation was noted between water hardness as measured by standard soap titration and the germicidal potency of the quaternary. Differences in hydrogen-ion concentration found in the natural waters examined had no significant effect on the quaternary. The cations calcium, magnesium and ferric iron decreased the germicidal potency of the quaternary, while potassium, sodium and lithium had no adverse effect. Ferric iron was considerably more detrimental than calcium or magnesium, which have similar effects on the quaternary. When the water contained as much as 1000 p.p.m. of calcium or magnesium, the 200 p.p.m. of quater-

nary was sufficiently potent to give approximately 100 per cent kill on *E. coli* after 8 minutes contact, while as little as 10 p.p.m. of ferric iron completely inactivated the quaternary. The anions studied were chlorides, sulfates, nitrates and carbonates, and no adverse effect was noted.

While the study is being continued, the results to date indicate that when the quaternary is added to most potable waters, a concentration of 200 p.p.m. has sufficient reserve germicidal potency for most sanitizing jobs.

M50 Germicidal Effectiveness of Certain Hypochlorites and Quaternary Ammonium Compounds under Simulated Plant Conditions.

P. R. ELLIKER, Oregon State College, AND K. R. SPURGEON, Purdue University.

Germicides employed in this study were selected by three screening tests. The hypochlorites selected included one highly active and one slower acting compound. The quaternaries selected included one widely used commercial compound and a second pilot plant product of comparatively higher germicidal activity. Plant germicidal procedures were simulated by controlled trials with stainless steel dishes and experimental 50-gallon cheese vats. Exposure periods varied from 15 seconds to 3 minutes. Concentrations of germicide varied from 25 to 200 p.p.m.

Exposure for short periods comparable to germicidal treatment just before use of equipment indicated more rapid germicidal action by the hypochlorites than by the quaternaries. The difference was slight in certain trials with pure cultures of *Streptococcus lactis* and thermophilic micrococci, but was quite pronounced in numerous trials with coliform species. The fast acting hypochlorite exhibited more rapid germicidal action in every case than either quaternary compound. Results suggested the desirability of raising the temperature of the quaternary solution for rapid destruction of coliform bacteria on dairy equipment.

A number of trials were undertaken to study residual or bacteriostatic effect of germicides applied to equipment after regular cleaning and rinsing at the end of the day's operations. Results indicated that where the equipment dried rapidly, treatment with hypochlorites resulted in fewer surviving bacteria. However, where equipment remained in a moist state, the continued bacteriostatic and bactericidal action of the quaternaries resulted in lower numbers than when hypochlorites were employed.

M51 Sanitizing Milk Cans in Mechanical Can Washers. G. W. REINBOLD, S. L. TUCKEY, R. V. HUSSONG, AND P. H. TRACY, University of Illinois.

M52 Some Factors Involved in Developing a Sediment Test for One-pint Samples of Cream Taken off the Bottom of the Original Container. BEN M. ZAKARIASEN AND RAY W. MYKLEBY, Land O'Lakes Creameries, Inc., Minneapolis, Minnesota.

New cream grading regulations in Minnesota have made it important that a rapid, accurate, and practical method be developed for making sediment tests on one-pint samples of cream taken off the bottom of the original container at the receiving room. A tentative method is presented, along with some of the data used in arriving at this procedure.

The tester used is the plunger type, having a one-quart capacity and graduated to hold one pint of diluting solution with one pint of cream. By using a diluting solution consisting of water at 150° F. for sweet cream and a 4 per cent sodium bicarbonate solution for sour cream, very satisfactory tests can be made. The method consists of drawing one pint of diluting solution into the tester, followed by one pint of cream, gently tilting tester to facilitate mixing of solution and cream, and forcing the solution through the lintine disc. The new tester and new heat-resistant lintine disc are described and shown. This method is being used in Minnesota as well as several other states and has been generally accepted in the routine operation.

M53 A Skunk-like Odor of Bacterial Origin in Farm-separated Cream.
T. J. CLAYDON, Kansas State College.

During quality studies on cream delivered by producers to buying stations in Kansas, a sample was obtained during the winter season that manifested a strong skunk-like odor. Such an odor has been recognized previously as a defect in commercial butter and has been attributed either to the development of *Pseudomonas mephitica* or to plants consumed by cows. The defect in question was found to be the result of associative action of two species of bacteria tentatively identified as a *Pseudomonas* species and *Streptococcus lactis*. These organisms, in combination, were capable of producing a skunk-like odor in milk, cream and butter. The development of the defect depended on the balance of organisms, growth temperatures and pH and also appeared to be affected by the extent of exposed surface of the product. Various objectionable odors, including rancid and putrid types, frequently developed.

Since the bacteria responsible for the skunk-like odor appear to be common in raw cream, the defect might develop in cream or butter when conditions contribute to a suitable balance of organisms. Both species of organisms readily are destroyed by heat and should not survive in properly pasteurized products.

M54 Coliform Bacteria in Butter. R. N. SINGH AND F. E. NELSON, Iowa State College.

The coliform counts on 294 samples of commercial butter were found to be related in only a very general way to the initial score or to the keeping quality of butter. Many butter samples had a coliform count of less than 2 per ml. Line run samples showed that salted butter with low coliform count frequently is obtained from cream which has been contaminated with considerable numbers of coliform bacteria. Coliform counts detected contamination early in the processing operations more accurately than did yeast and mold or total plate counts. Studies on three strains of *Escherichia coli* and two strains of *Aerobacter aerogenes* introduced in considerable numbers into cream before churning showed that strain of organism, amount of salt and temperature of storage all affected the coliform population of butter. The field of applicability of the coliform count for butter seems to be for use on line run samples to detect sources of contamination. Too many uncontrollable factors affect the coliform count of commercial butter samples to permit satisfactory use of the test for control purposes.

M55 The Effect of *Streptococcus lactis* and Coliform Organisms on Soluble Nitrogen in Milk. E. B. COLLINS AND F. E. NELSON, Iowa State College.

A study was made of the trichloroacetic acid-soluble nitrogen produced by four strains of *Streptococcus lactis*, one strain of *Escherichia coli*, and one strain of *Aerobacter aerogenes* growing separately and in combination. All cultures were isolated for the study, and only very active strains were used.

Inoculations of 0.1 per cent culture were made into skim milk which had been pasteurized for 20 minutes at 185° F. Cultures were grown at 30° C. in large-mouthed, screw-cap, 4-oz. bottles, and tests were made at 0, 1, 3, 7 and 15 days. Tests included soluble nitrogen, insoluble nitrogen, total nitrogen, titratable acidity, total plate count and coliform count.

Strains of *S. lactis* gave a small immediate rise in soluble nitrogen; which then continued to increase gradually throughout the 15-day test period until approximately 15 per cent of the total nitrogen was in the soluble form. The magnitude of the increase varied slightly with different strains of *S. lactis*.

E. coli and *A. aerogenes* caused decreases in soluble nitrogen during the first 1 or 2 days of growth. These deficits gradually were overcome, and with each organism more than 20 per cent of the total nitrogen was in the soluble form at 15 days. The maximum soluble nitrogen produced by *A. aerogenes* was greater than the maximum produced by *E. coli*.

The soluble nitrogen values resulting from the action of two strains of

S. lactis growing in combination with *E. coli* and *A. aerogenes* resemble an average of the values produced by the organisms growing separately, except that the values at 15 days were essentially the same as for *S. lactis* alone.

EXTENSION SECTION

E1 Report of Dairy Records Committee. C. R. GEARHART, Pennsylvania State College.

This four-part report will present the following points:

1. Status of Uniform Testing Outfit. J. F. Kendrick, Bureau of Dairying. The final report on the D.H.I.A. outfit was made last year, but there is additional information that should be mentioned.

2. Report on Results When Samples from One Milking are Used. C. T. Conklin, Sec'y., Ayrshire Breeders' Association. This is in the form of an investigation and will be a comparison of results from sampling one milking as compared with evening and morning samples.

3. Simplified Figuring in D.H.I.A. Work. J. D. Burke, Cornell University. This will deal not only with the results of short cuts or simplified figuring, but also with its effect on the supervisors and the attitude of the dairymen and breed associations.

4. Some Facts about Compensation Insurance, Health Insurance, Hospitalization, Social Security, and Income Tax Deductions for D.H.I.A. Supervisors. R. C. Jones, Bureau of Dairying. These facts will be presented in their legal aspects rather than how such practices are carried on in the individual states.

E2 Seven Years of Central Laboratory Testing. J. E. STALLARD, University of Wisconsin.

Seven years of production testing under the Central Laboratory Testing plan in Wisconsin have shown: (a) That more cows now are being tested than could be tested under the individual association plan and at less cost to both owner-sampler and standard type members. (b) That better work is being done in the laboratory than on the farm as to accuracy and volume. (c) That better book work seems to be the rule in Standard herds. (d) That testers can be paid larger salaries plus adequate travel allowance. (e) That testers prefer laboratory type to the old method. (f) That most old-type individual associations now are doing owner-sampler testing and quite a percentage of them have established individual laboratories in the homes of the testers.

Eight per cent of Wisconsin's producing cows now are being tested. Eighty-six per cent of the herds and 84 per cent of the cows on test are in cooperative central laboratory associations. Sixty-five per cent of the

herds and 59 per cent of the cows on test are on the owner-sampler plan. Fifty-nine of the 62 counties in Wisconsin having Dairy Herd Improvement Associations have central testing laboratories.

The major difficulties consist of finding enough testers or fieldmen, maintaining an adequate training program, and supervision in the field. Efficient systems, short cuts, and improved equipment are a result of this type of testing.

E4 Interdepartmental Cooperation on Dairy Extension. EVERT WALLENFELT, GEORGE WERNER, AND CARL NEITZKE, University of Wisconsin.

This paper describes the development, planning, and carrying out of an extension program through the cooperation of nine University departments involving thirty specialists. Other interested groups such as the Wisconsin State Department of Agriculture, American Dairy Association of Wisconsin, dairy manufacturing trade associations, Wisconsin Dairy Federation, Portland Cement Association, and electric power companies (both private and cooperative) participated.

Forty-eight county extension plans of work had milk quality improvement as one of their projects for major emphasis. Recognizing the need for more dramatic emphasis on the improvement of the quality of milk and dairy products, the Director of Extension appointed a University Dairy Quality Committee made up of members of nine University departments. This Committee was given the task of developing an integrated dairy quality program to fit the needs of the counties.

Among the first projects was the preparation of a series of exhibits for Farm and Home Week at Madison early in 1947. Many county agents who saw this exhibit asked that it be used at county fairs. The Committee felt that this exhibit was not well suited for fairs but that it might be developed as a traveling exposition to be shown during the winter months.

The success of this entire project is a striking example of what can be accomplished through cooperation of many groups in effectively planning and carrying out a far-reaching extension project. The interest stimulated by this exposition has set the stage for effective dairy quality follow-up work in the counties.

E5 New Methods of Selecting Calves for 4-H Dairy Club Work. RALPH PORTERFIELD, University of Maryland.

One of the main objectives in getting 4-H Dairy Club members started is that of locating good quality calves at reasonable prices. Several states have specific plans for selecting calves. In Maryland each breed association sponsors a calf selection event. It is a cooperative project involving

the breed associations and the extension service. Each breed association announces the date, place and time for holding the event. It also appoints a Calf Selection Committee and designates the price groups (*e.g.*, \$100, \$125, etc.). Price is based upon type, pedigree and age. The Extension Service makes a state survey among 4-H Club members to determine breed interest. This information is submitted to the respective Calf Selection Committee.

On the day of the event all calves are assembled at a central location and distributed by means of a drawing. Pedigree information is distributed to each club member. The club members inspect the calves in the forenoon and locate calves they prefer. In the afternoon they draw a number for position to select.

These events are referred to as "Ayrshire Calf Drawing", "Guernsey Calf Selection Day", "Holstein Heifer Party", and "Junior Jersey Jam-boree". A total of 120 purebred heifers have been distributed through these media for the 1948 Dairy Projects.

E8 Suggested Revision of D.H.I.A. Rules and Regulations. C. R.
(GEARHART, Pennsylvania State College.

The D.H.I.A. Committee has requested all states to review the Uniform Rules in the Supervisor's Manual (B.D.I.M.-Inf-26) and to express approval or disapproval. Suggestions for revision are requested. The committee will summarize the suggestions and, with the help of these suggestions, will present a set of rules for adoption or for further consideration.

THE FORTY-THIRD ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The American Dairy Science Association assembled in Hardman Hall at the University of Georgia, Athens, Georgia, on Monday, June 14, at 9:30 A.M. H. B. Henderson, local chairman, introduced President Harmon W. Caldwell of the University of Georgia, who gave the welcoming address.

Association President Paul W. Tracy then was presented and gave the following address:

THE PRESIDENT'S MESSAGE

As President of the American Dairy Science Association, I wish to take this opportunity to extend to our hosts the deep appreciation of all the officers and members for granting us the opportunity to hold the forty-third annual meeting on the campus of the University of Georgia. Some of you have come hundreds of miles to participate in these meetings, and I am sure none of you will be disappointed either in the caliber of the programs or the wonderful hospitality these fine people will show us. You have all observed the way in which every one on this campus is working towards the one objective of making our stay in Dixie a pleasant one. It is a big undertaking to house, feed and entertain a family as large as ours has grown to be, and I want you to know, President Caldwell, that our Society greatly appreciates your willingness to take us in. We are very grateful to Dean Chapman and Professor Henderson for the splendid work they have done in planning for our visit here. The cooperation of your faculty and physical plant staff in caring for our comforts and entertainment during the week is also deeply appreciated.

It was with some concern that I accepted the honor bestowed upon me when I was elevated to the presidency of the American Dairy Science Association. However, I found I was unnecessarily apprehensive about my responsibilities. Our Secretary, or more correctly speaking, Business Manager, Professor R. B. Stoltz, is so efficient that there is little for the president to do but sign his name to a few official documents. This is a fine tribute to the excellent work that has been done by our secretary over the period of years that he has held this important post. His annual report is further evidence that he is an excellent business manager of our association. I assure you that the affairs of the society are in good hands as long as we have a secretary-treasurer as loyal to our cause and as capable in his duties as Bob Stoltz.

Probably the most important project of the Society is our JOURNAL. When we have a good editor we want to keep him. It was for this reason

that two years ago the official board was reluctant to accept the resignation of T. S. Sutton. Some of you probably do not realize how much time is required to edit a good magazine twelve times a year. The Society was fortunate in securing an able successor to Professor Sutton. We greatly appreciate the fine job being done by Gene Nelson and we sincerely hope that he will be able to take enough time away from his family and professional duties to keep up the good work for a number of years to come.

The success of our annual meeting depends largely upon the seriousness with which the sectional officers and committee members take their responsibilities. I wish them to know that I deeply appreciate the fine work they have done during the past year in preparation for this program.

The job of supervising the organization rests pretty largely upon the official board. Your officers cannot make any important decisions without their approval. They are the first ones to arrive at the convention and are in session much of the time they are here. They give personally of their time, and I am sure that we all appreciate their efforts.

We Owe Much to the Founders of Our Society.—The first meeting of those interested in teaching and research in the field of dairying was called by the late Professor W. J. Fraser at the University of Illinois on July 17, 1906, while the Graduate School of Agriculture was in session at that institution. Action was taken at this meeting to form the National Association of Dairy Instructors and Investigators (later called the American Dairy Science Association) with a membership of eighteen. It is interesting to recall at this time some of the conclusions reached at this meeting 42 years ago.

For instance:

"1. Beet sugar causes sweetened condensed milk to ferment.

"2. The precipitation of both cane and beet sugar in sweetened condensed milk can be prevented by avoiding abrupt changes in the heating and cooling of the product.

"3. Acid silage will cause the formation of acid salts in the casein of milk.

"4. If large amounts of silage are fed there will be an increase in the acetic acid content of milk.

"5. In some sections of Chicago you will find 20-40% of the milk watered or preserved with formaldehyde."

Several needs were pointed out at this meeting such as:

"1. Knowledge of how to control moisture in butter and a quick method of determining moisture.

"2. More scientific knowledge on the subject of feeding cows.

"3. Better and safer market milk.

"4. Scientific knowledge of the principles involved in manufacturing condensed milk.

"5. Knowledge as to the food value of cheese."

One speaker pointed out that the text books on dairying contained many statements which lacked scientific proof as to their accuracy.

To these pioneers of our association we owe a great deal. They were naturally misinformed on some things just as we today no doubt have erroneous ideas regarding certain of our accepted scientific beliefs. However, they realized their shortcomings and were eager to learn the correct answers. It was this desire to learn and the resulting inspiration to younger men who had the privilege of being associated with the pioneers in scientific dairying, such as O. F. Hunziker, W. J. Fraser, C. H. Eckles, C. C. Hayden, E. S. Guthrie, C. E. Lee, and B. D. White, that have made possible the noteworthy advances in scientific dairying in this country. Let us never forget our obligations to those who founded our Society and laid the ground work for our present successes.

Scientists Should Help to Preserve Democracy.—There are a number of priceless heritages which we of the present generation have received from those who preceded us. Noteworthy among these is the faith that these men had in the future of dairying as an industry and the future of the American way of living.

Free enterprise, the profit system and unlimited resources have resulted in a national income in this country great enough to support a high standard of living for our population. This in turn has made possible the consumption of large quantities of milk, cream, butter, cheese, ice cream and other manufactured dairy products. Those of you who have traveled over the world either as civilians or members of the armed forces do not need to be reminded of the scarcity of dairy foods in most countries outside America. The future of dairying is very closely related to the future of democracy in the United States. There is no place for highly developed dairy enterprises in countries where all workers are regimented, where worker incomes scarcely supply the purchasing power needed to take care of the barest necessities of life, where the state owns or controls all land, where there is no free enterprise, and where the profit motive does not exist except possibly in the black market.

There is widespread unhappiness throughout the world. People lacking in economic security are easily influenced by disciples of political and social reforms who promise relief from the existing ills. It is unfortunate that the political leaders of the civilized countries of the world have made such limited progress in bringing about suitable working relationships between nations. How can permanent peace and security be secured for all peoples? No one, of course, has the answer, but surely there must be a way for intelligent people to work out the answers to these problems. Scholars have amassed a wealth of knowledge pertaining not only to the physical and

chemical properties of matter but also to the basic laws of human relations—political science, sociology and ethics. The physical scientists are being blamed by some people for the possible early destruction of civilization through modern warfare, but possibly it is only the scientifically trained men and women who have sufficient knowledge of the fundamental laws governing social relationships to lead this world to the economic security and spiritual and political freedom we all desire. This, it seems to me, is the most important challenge confronting all scientists today. We have a responsibility to society to be fulfilled, not only through our contributions to the specialized field of knowledge with which we happen to be associated but also by participation in efforts to secure good government and good living for all people. It is important that we continue to contribute to the stockpile of basic truths. But our efforts will be in vain if in our close attention to the assigned task we overlook the infiltration of the enemy whose aim is to sabotage the social and political freedom we have treasured for so many years in America. We should alert ourselves to these dangers and assume major roles in the fight to maintain freedom, not only in scientific research and teaching, but in our economic, social and political practices as well.

If the world ever needed leaders capable of sound thinking, it needs them now. We have in this country many organizations representing the various professional groups such as ours. Is there no way in which this tremendous force organized for the basic purpose of promoting the betterment of man through exact observation and correct thinking can be used to a greater extent by our political leaders in solving vital national and international problems? When a war crisis arises, the importance of scientists to the military is recognized immediately. Large numbers of scientists were drafted or invited into the inner sanctum at Washington to help win World War II. Possibly what is now needed is recognition by more of our political leaders that scientists might also be useful in helping to win the peace.

Type of Cooperative Action Important.—Much progress has been made in bringing about cooperative action between the various groups in the dairy industry. This is a commendable practice as long as the cooperative action is not directed towards the accomplishment of selfish motives. The group action of dairy farmers, processors and health officers in building trade barriers around their own community or state for the purpose of excluding the competition of those in the same industry located outside the chosen area does not promote a permanent type of prosperity in the industry. Nor does an organization whose purpose is to rob processors of a competitive type of dairy product, of their raw milk supply or of the market for their manufactured product promote the welfare of the dairy industry. Use of

political alliances to promote laws beneficial to dairying and dairy products, but detrimental to competitive industries, eventually makes enemies for the protected industry and gains friends for the competitor. All branches of the industry should work together for the common good of the entire industry with proper consideration of the welfare of the consumer. Laws proposed to regulate our industry should be carefully considered before passage. It should be kept in mind that the more selfish we are as an industry, the more difficulty we have in getting along among ourselves; and the more laws we have passed regulating our operations, the more rapidly we are approaching a public utility status in the dairy industry. If we are to maintain the good will and confidence of the consumer, our industry needs to pay less attention to the importance of proper alliance with pressure groups and work cooperatively to maintain our industry on a sound fundamental basis so that there can be no criticism of our objectives.

We need to work cooperatively to make possible the efficient production of higher quality milk, cream and dairy products. The chemurgy experts will need to be much smarter than they are today to take away the market for our products, provided we are in position to supply the consumer with cream, milk, butter, cheese and ice cream that is top grade and at a price he can afford to pay.

Dairy Farmer Entitled to Good Income.—The efforts of some organized dairy groups have been directed towards means of increasing the return to the dairy farmer for the milk and cream he produces. I do not question the honesty of the intent, but I do question the logic of methods directed entirely towards higher prices to the producer as the solution. There are a number of reasons why the farmer should be well paid for his labor. However, this reward should result from efficiency rather than from artificially fixed prices which bring about high consumer costs. We must not lose sight of the fact that it is the functions of both the producer and the distributor that contribute to the price the consumer must pay for milk, and both of these groups have a moral obligation to society to reduce this cost to a minimum. This obligation will become increasingly important as consumer purchasing power decreases and competition from milk product-substitutes becomes increasingly difficult to meet.

Milk and Cream Quality a Fundamental Problem.—Studies made at the University of Illinois and elsewhere have shown wide differences in the cost of producing butterfat on dairy farms. The founders of our society discussed in their first meeting the need for improving efficiency in dairy farming. Dairy Herd Improvement Associations which they pioneered have done much to encourage better breeding, feeding and management methods. Yet we still have too many "boarder cows." We still have too many two- and

three-cow cream producers. Extension forces of our colleges and the procurement managers of our dairy plants have much unfinished business ahead of them in the establishment of more efficient dairy farming. We need to bring about permanent cures where farmers are inefficient in the production of milk and where farmers are chronic violators of the sanitary principles of milk and cream production. These problems can be solved only by helping such farmers to appreciate the need for a complete reorganization of their master plan of farming. As Professor Fraser once emphasized, a farmer will be a good dairyman only when he has all his operations in balance. A few "pep talks" on high quality milk or cream production will be of little value unless the farmer knows and appreciates the importance of soil erosion, crop rotation, proper feeding and breeding methods, use of labor-saving devices, modern conveniences for his home as well as the milk house, application of sanitary methods in all phases of farm practices, and proper social and recreational programs for the members of his family. Dairy farming is not the easiest type of farming. While demand for milk is increasing, the amount produced in the United States is decreasing. This is a matter of great concern to processors and distributors of milk and its products. If we are to attract the right type of young people to the dairy profession and if we are to secure the milk needed to supply demand, we must encourage the general adoption of modern dairy farm practices and the establishment of more interesting living conditions for dairy farmers and their families. This will call for the cooperative effort of all branches of our university and industry extension services, not overlooking the role the rural sociologist should play in this program. An emotional approach to the problem is not sufficient. Young people today are realistic in their thinking.

Engineering to Play an Important Part in the Future Development of Our Industry.—In the evolution of the mammary glands of the dairy cow little consideration was given by nature to the fact that many thousands of years later man would have occasion to ship milk from one section of the country to another. As a result we are forced to heat, cool, package and transport millions of pounds of water each year in order to supply consumers with the milk solids they desire for nutritional purposes. We have attempted to correct this inefficiency by perfecting concentrated forms of milk, but with limited success. If all housewives would accept milk in the form of "evaporated," the cost of milk solids to the consumer would be materially reduced. However, until we can perfect a sterilized canned milk that does not have the objectionable cooked flavor, Mrs. Housewife will insist on being supplied with regular pasteurized milk. Theoretically, powdered whole milk should be the ideal product for the market. However, the consumer acceptance of this product will be in direct proportion to the success of our efforts to improve the flavor and physical consistency of the reconstituted

milk. The chemical or food engineer will play an important part in the development of new methods of processing milk and milk products which will improve quality and reduce marketing costs. It may be possible in the future to collect milk from farms only two or three times a week without damage to either the flavor or sanitary quality of the milk. We may be able to kill harmful bacteria and enzymes at the farm by some acceptable means other than heat. We may find it possible to condense milk at temperatures no higher than 60–70° F. and sterilize without any detrimental effect upon flavor. We may find it possible to perfect single-service containers that can be made from material even cheaper than wood pulp. By a highly developed system of mechanical devices it may be possible to dump, sample and weigh milk automatically. It might then flow automatically and continuously through the plant guided by a master control panel board. Cleaning and sterilizing may be done in the same manner. Similar mechanical perfections in the processing and packaging of butter, cheese and ice cream offer a fertile field for experimentation. Already some progress is being made along the lines suggested. The next few years may bring forth some startling innovations in the field of dairy engineering.

Problems in Teaching and Research.—The marvelous developments made in the physical, chemical and medical sciences during the past 10 years serve as a challenge to our industry. The huge sums of money made available by the government for research in these fields not only have attracted top-notch scientists but have made available the facilities of extremely well-equipped laboratories. Research workers in these basic fields are setting a fast pace for those of us working in the applied fields. To hold our own we will need more and better equipped laboratories and expertly trained men and women for these laboratories. There is a definite shortage of well-trained dairy research workers in the industry at this time. There is an inadequately supply of young men entering this field. To get better-trained research workers we will need to do one of two things—train our own or bring in students from the arts and science colleges. If we train our own, we will probably need to offer two types of curricula, one for the students not scientifically minded but who wish to return to the farm or prepare for work in industry, the other which will give the student very little training in the practices of dairying but will supply essentially a basic training in arts and sciences. Matters will not be so simple as this, however, as it may be difficult to get students to register in colleges of agriculture to take a basic science course. Most of the better students interested in basic science will very likely prefer to register in colleges where they can major in mathematics, chemistry, physics or medicine. Industry or government subsidy of promising students who can be induced to go into the applied scientific fields may help to solve the problem. At any rate, something must be done to better

acquaint good students with opportunities in the dairy field for those trained in fundamentals of the basic sciences.

Reorganization of our agricultural colleges may be another approach to this problem. For years departments, in some colleges at least, have been organized on the basis of the products of the farm, that is, dairy husbandry, animal husbandry, agronomy, horticulture and the like. Considering the extent to which basic research is moving in to supplement and to some extent replace applied research, possibly we should change our organization pattern to a functional type. In this type of organization we could have such departments as chemistry, bacteriology, engineering, economics, physiology, soils and nutrition, with the research and teaching emphasis directed towards the problems of agricultural pursuits. Most of the farm practice courses now taught in colleges could be delegated to the secondary schools and high schools.

There is great need for better laboratory facilities in universities carrying on teaching and research in the field of dairying. The day is past when research can be done with facilities limited to a Babcock tester, a herd of cows representing the common breeds of the state, a bomb calorimeter, a balance or two, a few microscopes, some petri dishes, a Kjeldahl apparatus, a churn, a freezer, a cheese vat, a pasteurizer and cooler, a milk bottler and a \$10,000 creamery balance to turn over to the University authorities at the end of the fiscal year. If dairy scientists are to do classical research, they will need as good tools to work with as will those engaged in basic research in the fields of chemistry, physics and medicine. If this is not done, basic research will be moved out of our laboratories into those better equipped.

The main agricultural problems confronting the world today are: (1) the increasing population, (2) the decreasing food supplies, (3) decreasing soil fertility and (4) need for supplying people with food that will maintain optimum conditions of health and vigor. These problems have social, economic and political ramifications and are extremely important to the future welfare and happiness of all people. The satisfactory solution of these problems will call for group action on the part of our best physical and social scientists. Dairy products make up 20 to 25 per cent of the food intake of this country. It is extremely important, therefore, that we organize our research program in such a way that we can contribute our share of the answers.

I wish to mention certain specific problems that may have some influence in shaping the future plans for research and teaching in the field of dairying. Scientific developments which might be used in the destruction of armies and urban centers have been so publicized that the mere mention of war gives us a creepy feeling. Yet, consideration may need to be given to such problems as the possible effects of radiant energy and biological warfare not only upon the body functions of the dairy cow but upon the quality of

the milk she secretes. The possibilities of this type of warfare may lead to the re-location of important food factories, such as large milk bottling plants and evaporated milk factories. Possibly decentralization of such food enterprises would add to the national security.

How will present trends in our social and political pattern affect our teaching and research programs? Will organization of farm labor raise production costs so that the price of milk will be pushed beyond the reach of many consumers? Will the complete organization of dairy plant workers prevent the college-trained men from entering the industry through the usual channel? Will government control of the buying and selling of milk work to the advantage or disadvantage of the industry as a whole? Should there be one quality standard for all milk regardless of its use? Would a return to whole milk creameries help restore butter to the popularity level it once enjoyed in this country? Will the future development of our industry depend upon our ability to devise means of producing and marketing milk and its products more cheaply or upon the results of basic research in the fields of biochemistry and human nutrition which may reveal facts regarding the food value of milk not before understood?

Will the centralizing of industry in the hands of a few large companies lead to more government control? How will the growing trend of big industries to promote their own research programs and train their own personnel affect the objectives of the college department, particularly in the field of dairy manufactures? What type of research should be done by industry? What should be our objectives in training college students? How can the field of dairying be made more attractive to capable young people deciding upon their life work? These are a few of the important problems that our leaders of today need to consider.

The need for food has been the driving force behind all races of men. It has been the controlling factor in the survival of all species of wild animals. It is the main cause of most of the unrest in the world. We have plenty of food in this country today. However, in order to protect our own standards of living, it is imperative that we take immediate steps to help the less fortunate people of the world secure for themselves proper food and living conditions. Greater food production, not only in America but the world over, is a must; otherwise continued struggle of one class or nation against those in other groups or countries will continue to sap our resources. Dairying is one of the most important of food industries. We must ever continue to exert our energies to bring about more efficient use of our lands in the production of milk of highest nutritional value, and we must continue our efforts to improve the methods followed in giving milk and milk products the necessary form and place utility. Progress in the science of dairy farming and dairy manufacturing must keep pace with progress in other scientific fields if our production is to survive in the social and economic order that lies ahead.

After a musical number by Prof. Robert I. Harrison of the Music Department, University of Georgia, the Dean of the College of Agriculture, Paul W. Chapman, gave a most interesting talk.

There were 402 members present.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Athens, Georgia, June 16, 1948

President Tracy called the meeting to order at 3 p.m. in Hardman Hall. There were 218 present.

REPORT OF THE EXTENSION SECTION

At the opening session of the Extension Section held June 14, chairman E. H. Loveland appointed for the Resolutions Committee, Heebink, Arnold and Reaves and for the Nominating Committee, Brownell, Copeland and Linn. A symposium was held on testing and dairy records with reports on standard testing equipment, simplification of records and responsibility of dairy herd improvement associations for various taxes and insurance.

At the second session an illustrated talk on interdepartmental cooperation on dairy extension was presented, followed by the presentation of Extension Exhibits on Teaching Methods. Twelve states presented exhibits in this feature of the program. The report of the Teaching Methods Committee, presented by I. L. Parkin, committee chairman, was adopted.

At the joint session of the Production and Extension Sections held Tuesday afternoon, a symposium on reproductive problems in dairy cattle was held. A report on the activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association was given by P. H. Phillips, and a brief talk on the program of the Purebred Dairy Cattle Association was made by G. A. Bowling, secretary.

The report of the joint committee on Dairy Cattle Breeding concerned recommendations for methods of sire proving and evaluation and a report on the new rules governing artificial breeding of purebreds. It also recommended the establishment of a joint Production and Extension Committee on type classification to give attention to problems in type as a factor in herd and breed improvement. The report was accepted as read. Enos Perry, chairman of the Dairy Cattle Breeding Committee, will send a copy of the complete report of this committee to each state within a short time.

Reports of the Breeds Relations Committee and of the Dairy Cattle Health Committee were read and adopted. (See report of the secretary of the Production Section.)

On Wednesday morning, 4-H club work was taken up as to national and regional contests, systems used in obtaining 4-H calves, and adoption of

practices as a result of 4-H dairy work. Joe Nageotte, committee chairman, acted as leader. In discussion of a national 4-H dairy judging contest, Heebink moved that the 4-H Committee for 1949 contact the 4-H Committee of the National Land Grant College Association relative to approval of a national 4-H contest. The motion was passed.

Charles Gearhart, chairman of the Dairy Records Committee, presented proposed changes for DHIA testing rules, which were taken up and discussed. A motion that the changes as revised in the meeting be adopted was made by J. D. Burke. The motion was passed.

At the business meeting, the report of the Resolutions Committee was read and adopted. Following the report of the Nominating Committee, Raymond Albrechtsen of New York was elected secretary for the coming year; other offices are to be filled by succession from secretary to vice-chairman and vice-chairman to chairman.

Gerald Heebink, incoming chairman, named the new members on the various committees and recommended that the 1948-49 committees hold a meeting before leaving Athens.

The recorded attendance was 79, with 32 states and provinces represented.

Respectfully submitted—E. H. LOVELAND, *Chairman*; GERALD HEEBINK, *Vice-chairman*; C. W. REAVES, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held eight sessions at which some 75 papers were presented. It was necessary to have two sessions running concurrently because of the large number of papers.

In addition, a symposium on "Reproductive Problems in Dairy Cattle" was held at a joint session of the Production and Extension Sections. Papers on "Infectious Diseases as a Cause of Infertility", by D. E. Bartlett, "Functional Causes of Infertility and Methods of Treatment", which included discussions of hormonal and nutritional aspects by S. A. Asdell and the effects of inheritance by L. O. Gilmore, and "Possible Modes of Approach to the Study of Infertility", by J. F. Sykes, were presented at this session. In addition, P. H. Phillips reported on the activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association. G. A. Bowling presented the program of the Purebred Dairy Cattle Association.

The following reports were presented and accepted by the Production and Extension Sections while in joint session.

The Report of the Breeds Relations Committee covered the activities of the committee in conjunction with the Purebred Dairy Cattle Association as related to "Uniform Rules for Official Testing". These rules have been revised and reprinted.

The Report of the Dairy Cattle Breeding Committee was read and accepted.

The symposium was sponsored by the Dairy Cattle Health Committee. The lack of fundamental information and the need for sound experimental studies were stressed in order to provide dairymen and research workers with adequate information on the prevention and correction of reproductive disorders. The need for continued research and educational activities in relation to the control and eradication of brucellosis, mastitis, calf losses and other diseases was indicated. Cooperation by the various interests is needed if rapid strides are to be made in the promotion of herd health.

The Committee on Resolutions of the Production Section consisted of Dwight Espe, R. E. Horwood and C. E. Wyle, chairman. The resolutions presented by this committee were read and approved.

The Nominating Committee of the Production Section consisted of D. M. Seath, I. R. Jones and K. L. Turk, chairman. Nominations for secretary were presented. L. O. Gilmore was elected. Vice-chairman L. A. Moore succeeds as chairman and secretary G. M. Cairns succeeds as vice-chairman.

The Report of the Dairy Cattle Judging Committee was presented by R. E. Johnson. He moved that the Production Section recommend to the American Dairy Science Association that the Dairy Cattle Congress at Waterloo, Iowa, be designated as the National Intercollegiate Dairy Cattle Judging Contest. This would be subject to the approval of the management of the show. The motion was duly seconded by D. M. Seath and approved.

It was voted to reactivate the Forage Committee to work with the related societies on problems in this field.

It also was voted to appoint three men to the Type Classification Committee that would work in conjunction with three men appointed from the Extension Section.

A motion made by K. L. Turk and seconded by C. Y. Cannon that the Program Committee of the Production Section abide by the rule adopted by the section several years ago to the effect that no author's name will appear on more than two papers to be presented at the section meeting was passed.

Respectfully submitted—G. H. WISE, *Chairman*; L. A. Moore, *Vice-chairman*; G. M. CAIRNS, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE MANUFACTURING SECTION

The programs of the Manufacturing Section were held in accordance with program published in the May issue of the JOURNAL OF DAIRY SCIENCE. Of the 55 papers scheduled, only two papers (M23 and 51) were not presented. A symposium, headed by K. G. Weckel, on certain phases of sanitation in the Dairy Industry also was held, with special papers being pre-

sented by representatives of industry, government and university research groups.

The business meetings of the section were held in accordance with the schedule, with Chairman P. R. Elliker presiding. Standing committee reports were received from the following standing committees: (a) Committee on Milk and five sub-committees; (b) Committee on Butter; (c) Committee on Products Judging; (d) Committee for Standardization of Tests for Dairy Alkalis and Methods of Reporting Results.

In addition to the adoption of reports, the following motions were made and passed:

1. That all standing committees be continued for the coming year.
2. That committee chairmen be encouraged to prepare their reports in suitable form for publication in the JOURNAL OF DAIRY SCIENCE.

At the final business meeting the following officers were elected: J. H. Hetrick, secretary; D. V. Josephson, the secretary of the past year, succeeds to the vice-chairmanship, and the vice-chairman for the past year, E. M. Barker, succeeds to the chairmanship for the coming year.

Respectfully submitted—P. R. ELLIKER, *Chairman*; E. M. BARKER, *Vice-chairman*; D. V. JOSEPHSON, *Secretary*

Upon motion duly seconded, the report was accepted.

EDITOR'S REPORT

The twelve issues of volume XXX of the JOURNAL OF DAIRY SCIENCE printed during 1947 consisted of 830 pages of original articles, 13 pages of Association announcements, 15 pages of program for the annual meetings, 31 pages of proceedings of the annual meetings, 81 pages of abstracts of papers presented at the annual meetings, 39 pages of indices, 50 pages of membership list, 192 pages of abstracts and 7 pages of miscellaneous. This makes a total of 1,258 pages, exclusive of the advertising sections and blank pages.

The material printed included 93 manuscripts (58 in the production field and 35 in the manufacturing field), 103 abstracts of papers presented at the Annual Meeting and 475 abstracts of literature appearing in the Abstract Section. Of the 125 papers submitted for publication during the year, 12 were rejected and 45 were on hand at the end of the year in various stages of processing for publication. The volume of material in all categories was greater than during the preceding year, indicating that a return to prewar volume of published material is only a matter of a relatively short time.

The editor wishes to express his appreciation of the assistance which members have given, in many cases anonymously, in the handling of the affairs of publication. Particularly to be commended is the thoroughness with which the majority of the reviewers have carried out their work in

helping to improve the quality of the manuscripts and the cooperative spirit in which authors have accepted the suggestions of the reviewers. Mrs. Phyllis McKimpson has been of great assistance to the editor, both editorially and in the handling of a multitude of details.

Arrangements for the reorganization of the Abstract Section had been virtually completed, to be effective on January 1, 1948, when negotiations with British DAIRY SCIENCE ABSTRACTS for some form of cooperation were reopened at their suggestion. In view of the uncertainties thus introduced, the editor deemed it inadvisable to make any changes in organization or to attempt to expand coverage until such time as the future policy of the Abstract Section was decided by the Journal Management Committee and the Board of Directors. A clear-cut decision for either expansion or termination is essential. Correspondence concerning relationships with the Milk Industry Foundation and the International Association of Ice Cream Manufacturers led to a decision to ask members of these organizations to meet with the Journal Management Committee for clarification of future policies.

The slowness with which some issues of our publication have appeared is regretted. The editorial office has gotten material to the printers on schedule and has returned all materials to the printers within the shortest possible time, frequently well under the time ordinarily scheduled for editorial operations. The hope is expressed that publication will resume normal schedule soon.

Four issues of the News Letter were sent to all members and student affiliates during the past year. While numerous favorable remarks have been made to the editor about this publication, the lack of response in the form of printable news items from the great majority of our members leads the editor to recommend the dropping of this project unless the members of the Association express a definite desire to the contrary and the heads of departments and others in similar positions are willing to assume the responsibility of sending in appropriate news items regularly.

Respectfully submitted—F. E. NELSON, *Editor*

Upon motion duly seconded, the report was approved.

SECRETARY-TREASURER'S REPORT

Membership and Circulation. The report of our membership and total circulation for the past year and the first half of 1948 excels any previous 18-month period, the total being 3,675 for 1947. Thus far in 1948 we have a circulation of 3,877, which is 200 more than the total for 1947. We are not bringing our association to the attention of all the dairymen in this country. There are at least 5,000 dairy scientists and commercial dairymen who would be members of the Association or subscribers to the JOURNAL if they were familiar with this opportunity.

During the past 2 years the cost of publishing the JOURNAL has increased almost 50 per cent, and the management does not believe it advisable to raise dues, subscriptions or advertising rates. We feel quite sure that if the members as a whole are interested in bringing the opportunity of membership and subscriptions to other dairymen who are seeking information, we can increase our membership sufficiently to meet the increased cost.

The cost in 1948 will exceed our intake, especially when we insert the cost of publishing our 10-year index, but in the past we have accumulated earnings for the express purpose of taking care of our JOURNAL when the pinch came. In one or more years from now, unless printing costs decrease or unless our circulation increases, we will be forced to increase our membership and subscription rates.

The following data are a summary of our gain and loss in members for the year 1947:

Membership, December 31, 1946	1583
Gain: New members, 1947	139
Former student affiliates	46
Total gain	185
Loss: Members resigned and delinquent, 1947:	
Delinquent	83
Resigned	16
Deceased	6
Total loss	105
Net membership gain	80
Membership total, December 31, 1947	1663

It is interesting to note where the new members came from last year. Eighteen states gained 84 per cent of all the new membership. The following is a list of the new members by states for the year 1947:

New York	22	California	6	Washington	4
Illinois	17	Minnesota	6	New Jersey	4
Wisconsin	17	Missouri	6	Texas	4
Ohio	12	Michigan	6	Dist. of Columbia	4
Pennsylvania	10	Massachusetts	5	Florida	3
Canada	8	Tennessee	5	Montana	3
Maryland	7	Arizona	5		

The stimulus that our meeting in Georgia has had has already indicated its effect on the membership for the first 5 months of 1948. We have had 140 new members in that length of time and South Carolina leads all states by submitting 19 new members. B. E. Goodale and J. P. LaMaster are to be congratulated on the splendid team work. Their names are signed on all 19 membership blanks. They solicited these new members. I am not

familiar with the letter of solicitation, but I am sure that they will be glad to send you a copy. The people in South Carolina are not any more dairy-science minded than they are in other parts of the states, but in South Carolina they have two men who have interested more dairymen in the Association.

H. D. Lindquist of Massachusetts also is to be congratulated for the ten new members from that state. Georgia also added ten new members. California and Florida tied for fourth with eight new members each. The following states have three or more new members each:

Ohio	Wisconsin	Iowa
Michigan	Indiana	Maryland
Kentucky	Texas	Louisiana
Rhode Island	Oregon	Alabama

One more individual I would like to mention is M. H. Campbell of Rhode Island. He submitted four new memberships this year, thus doubling the membership in Rhode Island. Before Campbell went to Rhode Island, they had only two members.

In the first 5 months of the year, 17 states have submitted 100 new members out of a total of 140, or 71 per cent. We trust that these 17 states will continue their membership drive, and we also trust that the other 32 states will make an effort to increase their membership and number of subscribers in their respective states. This is the first year the Association has been able to take new members and supply them with back Journals after July 1. We are expecting to receive an additional 200 members and subscribers before the end of the year.

Student Branches. At the present time we have 16 student branches of the American Dairy Science Association at the following schools:

University of California	University of Missouri
University of Connecticut	Washington State College
Cornell University	Ohio State University
University of Florida	Oregon State College
University of Georgia	Pennsylvania State College
Iowa State College	University of Tennessee
University of Kentucky	Texas Tech College
University of Massachusetts	Virginia Polytechnic Institute

This year the Executive Committee granted certificates to the following schools: Alabama Polytechnic Institute, Michigan State College, and Montana State College.

Back Copies. Last year the income from back copies decreased from \$2,033 to \$1,880. Thus far in 1948 the figures show that our back copy sales will continue to decrease. The Association has replenished its stock of some of the early journals during the past year by purchasing them from members. This amounted to \$187.50. Our present inventory shows that

we do not have complete volumes of nos. 11, 29 and 30. By reproducing six numbers of back copies we would be able to furnish all back numbers. We recommend that they should not be reproduced until printing costs decrease. Our inventory shows the Association has on hand 31,392 individual journals in addition to the 20-year index.

Advertising. The total income from advertising in 1947 did not increase over 1946; however, we had 198 pages in 1947 versus 188 in 1946. In 1938 our present rates were set, at which time we had a circulation of 2,200 journals per month. Now we are printing 4,400 journals, or just twice as many, and are charging the same rate per page. We recommend that we increase our advertising pages by about 25 per cent this year and next so that we may bring our income from advertising up to about \$10,000 per year. We are pleased to take this opportunity to again express our grateful thanks to the organizations that use our journal as an advertising medium. Any courtesies shown our advertisers will be greatly appreciated.

Financial. The financial picture for 1948 is not encouraging. Our estimated expenses, due largely to increased printing costs and increased papers published, will amount to about \$35,500. Our estimated income is only about \$30,000. We are facing this year's financial situation with the thought of losing money and drawing on our reserve. In 1947 we had an income of \$28,221.06. This was an increase of more than \$2,000 in excess of the previous year. Our expenses for 1947 also excelled any previous year by almost \$5,000. They amounted to \$26,029.36, leaving an operating profit of \$2,191.70 on December 31, 1947. Our net worth on December 31, 1947, was \$38,085.21. Our investment in government bonds was \$36,287.00. A complete report of the Certified Public Accountant was sent to each member of the Executive Committee on March 10, 1948.

The Secretary wishes to take this opportunity to express his gratitude and thanks to the officers and members of the organization for the splendid cooperation that they have continued to give him.

Respectfully submitted—R. B. STOLTZ, *Secretary-Treasurer*

Upon motion duly seconded, the report was approved.

AUDITING COMMITTEE REPORT

The president then requested the report of the Auditing Committee, which was given by D. V. Josephson.

April 23, 1948

To the Directors and Members of the
American Dairy Science Association

Gentlemen:

On April 19, 1948, the Auditing Committee of the American Dairy Science Association met with Mr. Walter C. Burnham, a certified public

accountant who had examined the financial statement submitted by our secretary-treasurer.

Mr. Burnham reported after a thorough examination of the records, bank statement and checking account that all were accurate and in good order.

The Auditing Committee is satisfied that the financial statement for the year 1947 is correct and recommends that it be accepted by the Board of Directors and the members of the American Dairy Science Association.

Respectfully submitted—R. A. LARSON, W. J. BRAKEL, D. V. JOSEPHSON

Upon motion duly seconded, the report of the Auditing Committee was accepted and ordered filed.

REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

In order to make the JOURNAL OF DAIRY SCIENCE better serve the membership and still come within the cost permissible without increasing subscription rates, the following recommendations are made:

1. That the Abstract Section be expanded to more thoroughly cover the dairy literature.
2. In order to economize on the cost, that: The Abstract Section and membership list be published in double column and reduced type size.
3. Due to the apparent lack of interest in furnishing the editor with news, that the News Letter be dropped.
4. That the editor be empowered to subscribe to, or negotiate exchange of, the JOURNAL OF DAIRY SCIENCE for foreign dairy journals, the value of which is not to exceed \$75.00 annually.
5. That the Journal Management Committee select associate editors on a staggered 4-year term basis—two to be chosen each year and no one eligible for appointment for two consecutive terms.

Respectfully submitted—P. R. ELLIKER; T. S. SUTTON; R. B. STOLTZ.
Ex-officio; F. E. NELSON, *Ex-officio*; W. E. PETERSEN, *Chairman*

Upon motion duly seconded, the report was approved.

REPORT OF NATIONAL RESEARCH COUNCIL

During the past year, Robert F. Griggs, who served so ably as chairman of the Division of Biology and Agriculture, retired from that position. J. S. Nicholas, Sterling Professor of Biology, Yale University, is the new chairman.

In his opening address before the recent annual meeting of the Division of Biology and Agriculture, Dr. Nicholas urged that the member societies become more militant in pushing activities in their fields. He cited the situation with regard to the two and one-half million dollars which has been assigned by the Atomic Energy Commission for fellowships in five different areas, two of which deal with Biology and Agriculture. There were only 19 applicants for fellowships in biology and agriculture while there were 91

applications for training in the physical sciences. He suggested that this imbalance in applications for fellowships may be related to the higher salaries now being paid the physical scientists when compared to the biologist.

Dr. Nicholas also pointed out that the Veterans' Administration has requested the Division to investigate the agricultural training now being given under the G. I. Bill and make recommendations to them about it.

The announced policy of the Division is to put more emphasis on short-term projects which will give quick returns; although long-time projects seeking basic facts have great value, they do not attract funds as readily as do the short-term projects.

The Division through its Agricultural Board attempts to make essential contributions towards the solution of vital problems of agriculture. This is done through the activities of selected committees. The problems relating to dairying that are being studied at present are the "Public Health Aspect of Brucellosis", "Losses among Calves" and the "Dangers of Importing the Virus of Foot and Mouth Disease in Meat and Other Animal Products".

Already many of the members of the Dairy Science Association are acquainted with the objectives and organization of the American Institute of Biology, which was formally established on February 20, 1948, with twelve societies accepting full membership and one society adhering as an affiliate member. The National Research Council sponsored this organization of the Institute with the hope that it would function in the public relations field, where the Division as it now is organized cannot enter. Naturally, the hope is that all societies which now are affiliated with the Division of Biology and Agriculture in the National Research Council eventually will join the Institute of Biological Sciences to give it strength to fulfill its objectives.

Respectfully submitted—C. Y. CANNON

Upon motion duly seconded, the report was approved.

NECROLOGY COMMITTEE

K. M. Renner, Professor and Head of the Department of Dairy Manufactures, Texas Technological College, Lubbock, Texas, died September 2, 1947, following a cerebral hemorrhage. He received his B.S. degree from Iowa State College in 1921, and after serving on the Dairy Department staff at Kansas State College until 1927, he received his M.S. degree at Kansas and joined the staff at Texas Technological College, heading the Dairy Manufactures Department there until his death. He was active in civic, social, scientific and religious organizations. He is survived by Mrs. Renner, one son, two daughters and one brother.

Alan Leighton was born in Concord, New Hampshire, March 26, 1890, and died at Cottage City, Maryland, on February 11, 1948. He graduated from the University of New Hampshire and took graduate work at Cornell University. He was associated with the Bureau of Dairy Industry, USDA,

for 27 years before his retirement in 1947. Prior to his association with the Bureau, he was with the Bureau of Mines in Denver and Pittsburgh. He was very active in civic affairs, serving as town commissioner and fire marshal of Cottage City. He was widely known for his research and survey of literature on ice cream. Surviving are Mrs. Leighton, one son, and one daughter.

Ivan McKellip, Professor of Animal Husbandry at Ohio State University for 32 years, died after a month-long illness in December, 1947. Prof. McKellip was graduated from the University of Nebraska, received his Master's degree from Cornell University and taught at Massachusetts State College and later at Purdue University. He was widely known as a judge of dairy animals and was most active in 4-H club work. He is survived by Mrs. McKellip, six sisters, and five brothers.

George Girrbach, Sault Ste. Marie, Michigan, was born in Minnesota in 1890. He died May 24, 1948, following an auto accident while en route home from attending a special session of the Michigan Senate, of which he was a member. Mr. Girrbach was graduated from the University of Minnesota and received his Master's degree from Michigan State College in 1927. He served as Dairy Extension Specialist at Michigan State College from 1924 to 1930, after which he was owner and manager of the Soo Creamery, Sault Ste. Marie, Michigan. He is survived by his wife, Mrs. Ethel Girrbach.

Helmar Rabild was born in Denmark on August 10, 1876, and was naturalized in 1905. He graduated from Denmark Agricultural College, was an instructor at Michigan Agricultural College from 1902 to 1906, and was associated with the Chesterfield Creamery in Michigan from 1902 to 1903. He was Deputy Dairy and Food Commissioner in Michigan from 1905 to 1907 and in charge of dairy extension at the Dairy Division of the USDA, Washington. He organized the first cow-testing association in the United States. At the time of his death, on January 1, 1948, he was president and manager of the Titusville Dairy Products Company, Titusville, Pennsylvania. He is survived by Mrs. Rabild.

Weston A. Price of Santa Monica, California, died on January 23, 1948. Information concerning his death was received too late for publication.

Respectfully submitted—H. P. DAVIS; E. O. HERREID; G. M. TROUT

Upon motion duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE

WHEREAS: The University of Georgia through its administrative staffs and faculty has made available to the American Dairy Science Association in this its 43rd Annual Meeting all needed physical facilities for the meeting, and

WHEREAS: Every possible personal courtesy has been given to members of the Association for their enjoyment and entertainment,

Therefore, be it *Resolved*: That the American Dairy Science Association take this opportunity officially to extend its thanks and appreciation and hereby request the President of this Association to convey by letter this appreciation to President H. W. Caldwell and to Dean Paul W. Chapman and Prof. H. B. Henderson.

WHEREAS: The Borden Company has again offered its awards for outstanding research in dairy manufacturing and production,

Therefore, be it *Resolved*: That the American Dairy Science Association express to the Borden Company its sincere appreciation of this evidence of its continued interest in dairy research.

WHEREAS: The American Feed Manufacturers Association has seen fit to offer an award for outstanding research in the field of dairy cattle nutrition,

Therefore, be it *Resolved*: That the American Dairy Science Association express to the American Feed Manufacturers Association its sincere appreciation for their interest in and encouragement of research in dairy cattle nutrition.

WHEREAS: The Purebred Dairy Cattle Association has continued in its cooperation with the American Dairy Science Association in establishing uniform rules for the testing of dairy cattle, for the regulation of artificial breeding and other matters promoting uniformity and,

WHEREAS: The Purebred Dairy Cattle Association has established a Dairy Cattle Breeding Research Foundation for the purpose of encouraging and supporting research in this field, in cooperation with the various experiment stations,

Therefore, be it *Resolved*: That the American Dairy Science Association commend the Purebred Dairy Cattle Association for its efforts.

WHEREAS: Brucellosis is a serious disease of growing magnitude and concern to rural people, packing-house workers and veterinarians, and

WHEREAS: This disease affects cattle, swine and goats and causes great financial losses to the dairy and livestock industries,

Therefore, be it *Resolved*: That the American Dairy Science Association urges intensified brucellosis eradication activity throughout the country (at this time, particularly among cattle) with the eventual goal being brucellosis-free herds, counties and states.

Be it further *Resolved*: That all known and effective methods of control and eradication shall be used as individual state disease situations demand, and that additional research to find still better methods of control be further stimulated and encouraged to achieve the above objectives.

Be it further *Resolved*: That the Committee of the National Research Council under the chairmanship of W. W. Spink, appointed to investigate and study the relationships between brucellosis in humans and farm animals, be given the Association's encouragement and that the National Research

Council be urged to give the necessary aid and support for its continuance. A copy of this resolution shall be sent to the chairman of the Division of Biology and Agriculture of the National Research Council.

Respectfully submitted—S. L. TUCKY; W. A. KING; E. L. FOUTS

Upon motion duly seconded, the report was adopted.

REGISTRATION COMMITTEE

H. B. Henderson, University of Georgia, made the following report for the Registration Committee. Upon motion duly seconded it was accepted.

Alabama	9	Minnesota	16	Tennessee	14
Arizona	2	Mississippi	4	Texas	6
Arkansas	2	Missouri	11	Utah	1
California	4	Montana	1	Vermont	8
Connecticut	7	Nebraska	6	Virginia	8
Delaware	1	New Hampshire	4	Washington	4
Florida	12	New Jersey	11	West Virginia	5
Georgia	40	New Mexico	1	Wisconsin	37
Illinois	45	New York	40	Washington, D. C.	18
Indiana	7	North Carolina	18	Canada	5
Iowa	16	North Dakota	1		—
Kansas	7	Ohio	38	Total members	530
Kentucky	41	Oklahoma	5	Non-members	70
Louisiana	7	Oregon	4		—
Maine	1	Pennsylvania	14	Total	600
Maryland	17	Rhode Island	4	Women and children	356
Massachusetts	5	South Carolina	8		—
Michigan	15			Total attendance	956

Fordyce Ely moved and W. E. Peterson seconded that all action of the Executive Committee during the past year be approved.

MEETING OF THE EXECUTIVE COMMITTEE

AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The Executive Committee transacted the following business:

Approved the Minutes of the past Annual Meeting.

Approved the Editor's Report.

Approved the Secretary-Treasurer's Report.

Approved the report of the Auditing Committee.

Approved the budget for 1948 amounting to \$35,500.

Received the report of the representative of the National Research Council.

Re-employed the Editor and Secretary for the ensuing year.

Voted unanimously to make A. A. Borland an Honorary Member.

Elected G. H. Wise as a member of the Journal Management Committee to serve for the ensuing three years.

Voted to not affiliate with the International Dairy Federation.

Adopted the Journal Management Committee report.

Approved the Resolutions Committee report.

Voted to make Robert S. Breed a life member of the Association.

Approved the motion that the budget include the cost of travel by first class in a public conveyance for each member of the Executive Committee not now provided for to attend the annual meeting.

Accepted the proposal by W. E. Petersen that the Association meeting be held at the University of Minnesota on June 20 to 22, 1949.

The Nominating Committee, consisting of G. H. Wilster, F. J. Doan and G. C. North, nominated the following officers in April: Vice-president, W. V. Price of Wisconsin, and G. M. Trout of Michigan; directors, E. E. Heizer, Wisconsin; H. B. Henderson of Georgia; P. R. Elliker of Oregon; and C. A. Iverson of Iowa.

The results of the election were announced on June 1 as follows: G. M. Trout, vice-president; H. B. Henderson and P. R. Elliker, directors.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

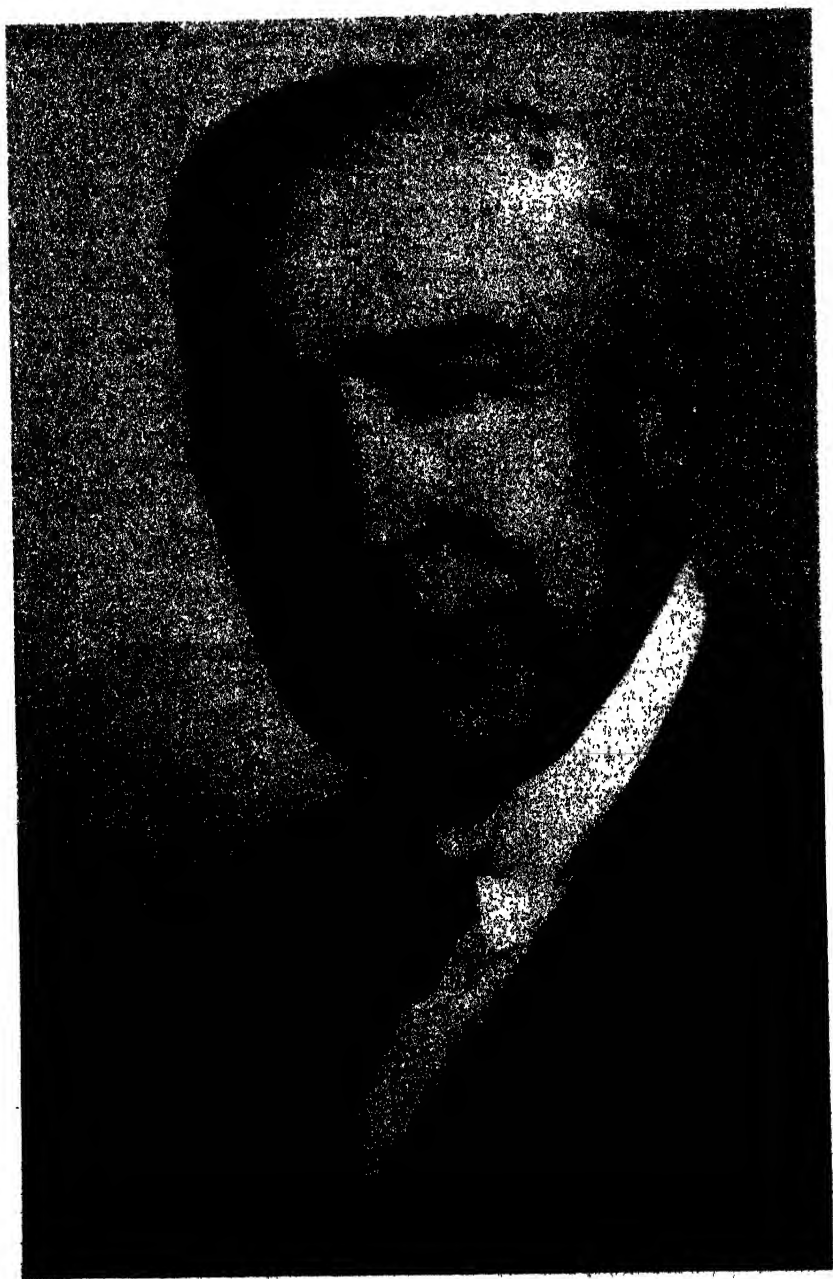
Athens, Georgia, June 16, 1948

H. B. Henderson, of the University of Georgia, acted as toastmaster at the barbecue and presented President P. H. Tracy, who installed the officers-elect as follows: W. E. Petersen, of Minnesota, was installed as President; G. M. Trout, of Michigan, as Vice-president; H. B. Henderson, of Georgia, and P. R. Elliker, of Oregon, as Directors.

Mr. Petersen, you are about to take over the responsibilities of President of the American Dairy Science Association. As President it will be your duty to be chairman of the Executive Board and submit to the Board for approval the nominations of members to fill vacancies that may occur among the elected officers of the Association. As President you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now charge you with the honor of being President of the American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto.

Mr. Trout, you are about to take over the responsibilities of Vice-president of the American Dairy Science Association. As Vice-president, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Petersen's term, you will automatically become President of this Association. I now charge you with these duties.

Mr. Elliker and Mr. Henderson, you were elected to the Executive Board of the American Dairy Science Association. It is the duty of the Board



ANDREW ALLEN BORLAND

members to pass on all applications for the establishment of divisions, sections and student branches of the Association. You will have full control of the budget and general business of the Association and have title to all property and funds of the Association. You will be members of a Board that has all the rights and power vested in the by-laws of the Association. With these privileges, responsibilities and obligations you are now considered as members of the Executive Board of the American Dairy Science Association to serve a term of three years.

PRESENTATION OF ASSOCIATION AWARD

The toastmaster then introduced A. C. Ragsdale, chairman of the Association Honors Committee, who made the following citation:

Andrew Allen Borland was born at Sandy Lake, Pennsylvania, married Jesse E. Canon, and has two children, Gerald Canon and Margaret Eleanor. He taught in the public schools, Mercer County, Pennsylvania from 1898 to 1905, graduated from Pennsylvania State College in 1909, and received the Master's degree from the University of Wisconsin in 1910.

Mr. Borland served as assistant in Dairy Husbandry research, Pennsylvania State College from 1910 to 1911; as professor and head of the Animal and Dairy Husbandry Department, University of Vermont, from 1911 to 1915; as professor in charge of Dairy Husbandry extension, Pennsylvania State College, from 1915 to 1919; and as professor and head of the Dairy Husbandry Department, Pennsylvania State College, since 1919, from which position he retires June 30, 1948.

He served as a member of the Board of Education, Burlington, Vermont, from 1913 to 1915; and has been a member of the Board of Civic Planning, State College, Pennsylvania, since 1930; a trustee of the Westminster Foundation, State College, Pennsylvania, since 1939; vice-president of the Westminster Foundation, State College, Pennsylvania, since 1941; served as president of the Pennsylvania Dairymen's Association, 1925 to 1927; and as president of the College Feed Conference Board in 1931.

Mr. Borland served as Vice-president of the American Dairy Science Association, from 1920 to 1922; president of the American Dairy Science Association from 1922 to 1924; president of the Eastern Division of the American Dairy Science Association in 1925; a member of the Journal Management Committee, from 1932 to 1944; chairman of the Journal Management Committee from 1943 to 1944; contributing editor, *Pennsylvania Farmer*, since 1923; United States and Pennsylvania delegate to the 8th World's Dairy Congress, London, Reading, Edinburgh, Glasgow, 1928; and as U. S. judge, 4-H International Dairy Cattle Judging Contest, Kent, England, 1928.

He has been an elder in the Presbyterian Church for many years and active in church affairs. He is a member of the Masons, Phi Kappa Phi, Gamma Sigma Delta, Delta Sigma Rho, Alpha Zeta, and Delta Theta Sigma.

To select his most important contribution is a difficult task. In the field of research, he has given generously of his time as an advisor to his colleagues. We make special reference only to his leadership in the research project on "Input and Output Relationships in Milk Production" which he conducted in cooperation with the United States Department of Agriculture. He will be best remembered as an outstanding teacher and able administrator. As a teacher, he has been deeply interested in character building. His interest in people and their love for him has been evidenced in his extension activities, the deep affection of his students and his participation in church and civic affairs. The dairy industry owes him much for his outstanding leadership throughout the years.

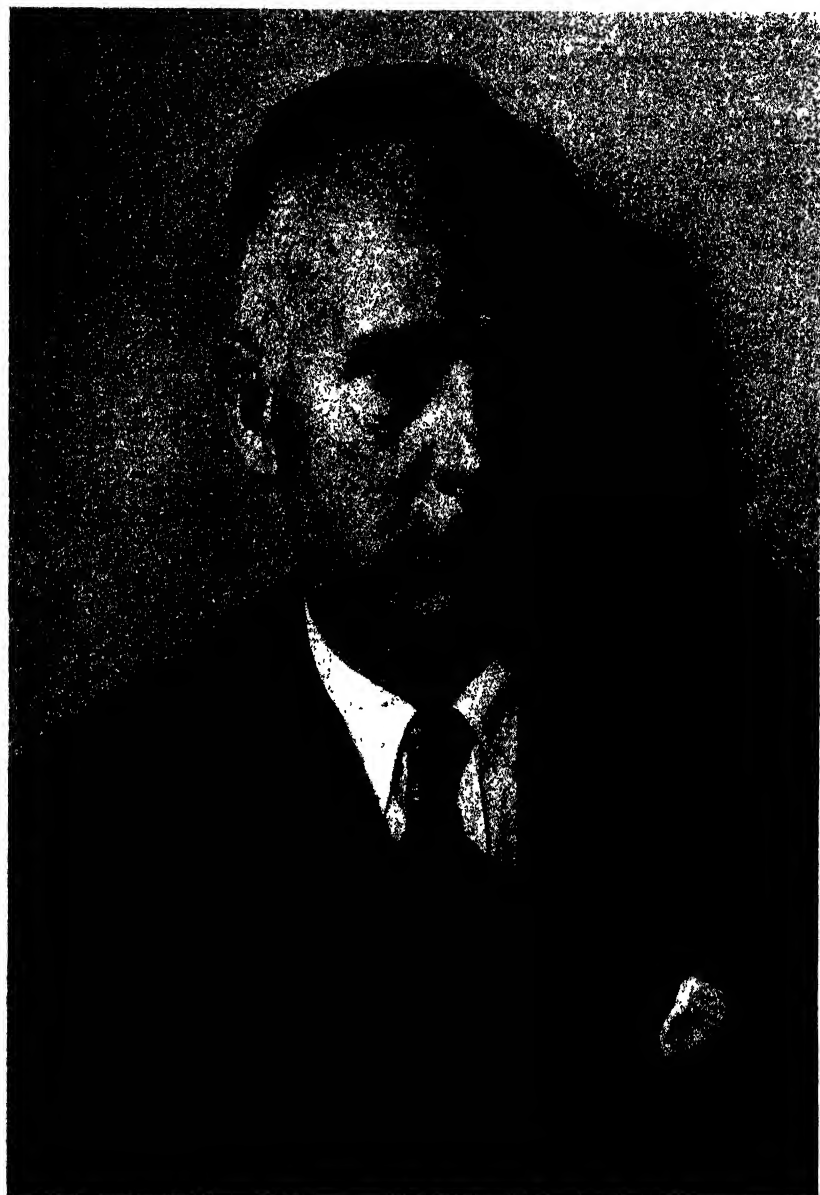
It is with great pleasure and appreciation for his life of service that we present to Andrew Allen Borland this Certificate of Honorary Membership in the American Dairy Science Association.

PRESENTATION OF BORDEN AWARDS

B. E. Horrall, chairman of the Borden Award Committee for Manufacturing, was then introduced and made the following statements:

The recipient of the Borden Award in Dairy Manufactures for this year is one of the leading dairy scientists and has made many outstanding contributions in the field of Microbiology and Chemistry of dairy products. Most of his research has been in the fields of butter and cheese. He has made extensive studies on the surface taint of butter and isolated many organisms that cause the many off flavors in butter. He has made timely studies on surface bleach in butter, the cause of wood taint in storage butter and treatment of butter boxes for its prevention. His work on mold and yeast problems in butter has gained wide recognition. In his cheese research he has published many papers, some of which are studies on starter problems, effect of copper on lipase activity, development of rancid and unclean flavors, use of clarified milk in cheddar cheese manufacture, and development of lipolytic flavor defects in cheddar cheese. He developed the "Pink Test" for determining setting-time in cheddar cheese-making. These and many other of his research studies have definitely given the candidate an enviable record among the outstanding dairy scientists of the world.

He received his Bachelor's degree from Ontario Agricultural College and his Master's and Doctor's degrees from Massachusetts Agricultural College in 1913, 1919 and 1922, respectively. Besides these degrees, in which he majored in Bacteriology and minored in Chemistry, he spent some time in the U. S. Army Medical Corps, six months of which were spent at Yale taking Medical Bacteriology, after which he was posted at Army Base Hospitals at Houston, Texas, New Haven, Conn., and Chicago, Ill. During his stay at Massachusetts Agricultural College he also did much research work on black smut in canned lobsters.



EDGERTON GIBSON HOOD

It was the unanimous decision of the Borden Award Committee for Dairy Manufacturing that Dr. E. G. Hood be selected as the recipient for the Borden Award this year.

In the fall of 1923 Dr. Hood was appointed Chief of the Division of Dairy Research under the Dairy and Cold Storage Branch of the Dominion Department of Agriculture at Ottawa. He has been in charge of Dairy Research work since that time.

Since joining the Dominion Department of Agriculture, most of Dr. Hood's work has been directed towards the study of major problems confronting the dairy industry in Canada. Dr. Hood has become well known in dairy circles in Canada and the United States, not only for his research but also through his attendance at dairy conventions and his connection with the American Dairy Science Association, of which he served as director for several years.

Mr. Wentworth, it gives me great pleasure to present to you Dr. Egerton Gibson Hood as recipient for the 1948 Borden Award in Dairy Manufacturing.

Mr. Wentworth then presented to Dr. Hood a gold medal and a check for \$1,000.

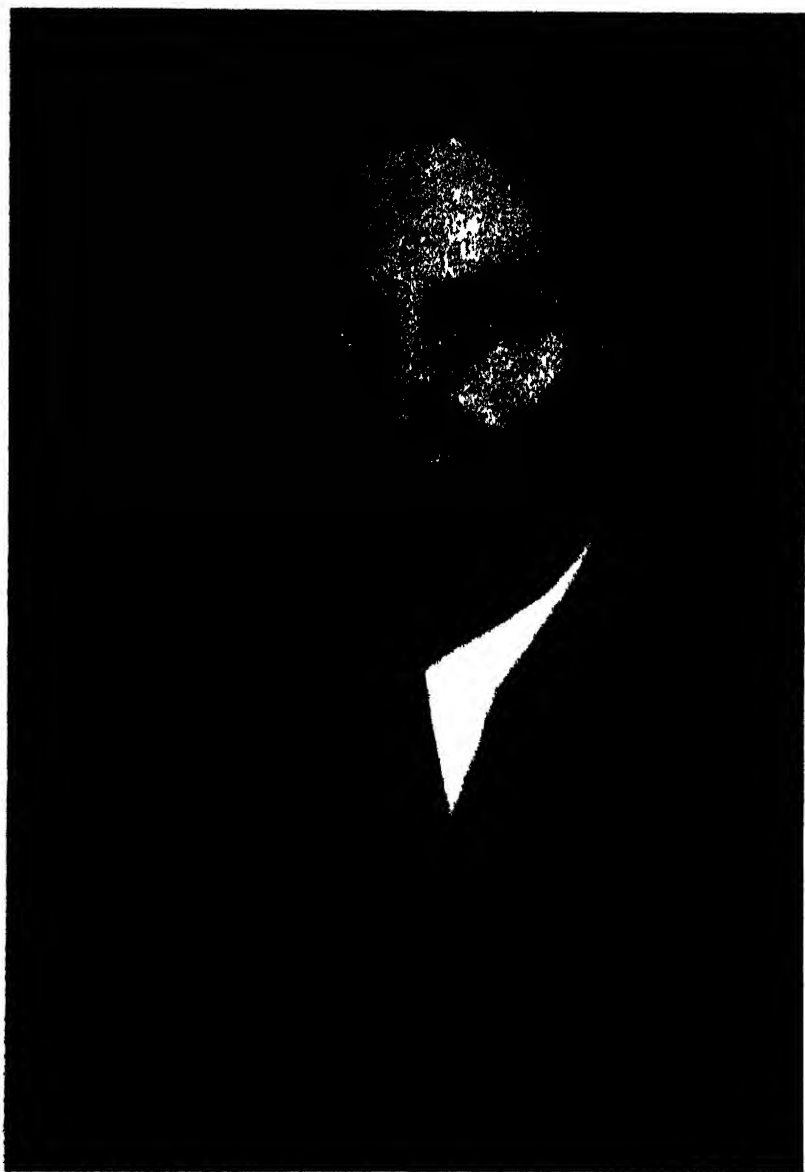
BORDEN AWARD IN DAIRY PRODUCTION

C. W. Turner, chairman of the Borden Award Committee for Production, then was introduced and made the following statement:

The recipient of the 1948 Borden Award in Production chosen by your committee has completed 40 years of intensely productive research at the University of Illinois. He is a native of that state, having been born at Crete, Illinois, in 1881. He was graduated with a B.S. degree from the University of Illinois in 1908 and received his Ph.D. degree from the University of Chicago in 1915.

His doctor's thesis, entitled "A Contribution to the Physiology of Lactation," published in 1915, not only set the pace for the high standards of excellence which he has maintained over the years in his publications but indicated the field of scientific interest which he has steadfastly pursued. He has been the modest leader in the United States of the scientific approach to the problems of milk secretion. He was one of the first to apply physiological methods to the problem of the "let down" of milk. His study of the quantity of milk present in the udder of the cow at milking time followed. Other early classic studies included the "relation between percentage fat content and yield of milk," "feed cost of milk production as affected by the percentage fat content of the milk," and "relative rates of secretion of various milk constituents."

These investigations culminated in the report on the "energy basis for measuring milk yields in dairy cows." The formula proposed for the



W. L. GAINES

equalization of milk yield upon the basis of the energy content of the milk has gained universal acceptance throughout the world. Fat-corrected milk (FCM) is standard practice in dairy cattle nutrition work as well as in milk secretion studies.

To the problem of the factors influencing the lactation curve of dairy cattle was applied not only a keen understanding of lactational physiology, but also mathematical and inventive genius as well. Slide rules and intricate mechanical equipment were designed to assist in the enormous task of fitting equations to the individual cow's lactation curve included in the extensive studies conducted over a period of years. Not only did his work point to the importance of persistency in the lactational performance of dairy cattle but the extent of the influence of pregnancy upon milk yield was accurately determined.

Measures of the efficiency of dairy cattle and the relation of body weight thereto next engaged the attention of this investigator. More recently the problem of the energy-size basis of measuring milk yield has been explored. Some 46 technical papers and bulletins have come from his pen. However, a mere enumeration of numbers of publications cannot indicate the numerous contributions of a truly fundamental character. One fellow investigator has said that "his work on the energy aspects of milk constituents is known around the world and quoted in writings in other lands perhaps more widely than that of any other American worker in dairy science."

One phase of his character less easy to evaluate has been his influence upon younger investigators in exemplifying the proper attitude, spirit and methods of the research worker. It has been said of him that "he has been cautious, skeptical in the best scientific sense of the word, always eager to get the actual evidence concerning any point under discussion, industrious, full of eager scientific curiosity, patient and gentle but firm in discussions and controversies with younger men. Many a younger worker has gone away from scientific meetings which he attended, having his thoughts clarified, any feeling of personality in the controversy removed, and with renewed eagerness to do some really sound work in advancing still farther the frontiers of knowledge in dairy science."

On behalf of the committee on the Borden Award in Dairy Production, it is a very great personal pleasure and an honor to present Dr. W. L. Gaines, Professor and Chief in Milk Production of the Illinois College of Agriculture, to receive the Award.

Mr. Wentworth then presented Dr. Gaines a gold medal and a check for \$1,000.

R. B. Becker, acting chairman of the Award Committee for the American Feed Manufacturers' Association, then was introduced and made the following statement:

Early in the year, the American Feed Manufacturers' Association de-



GEORGE HERMAN WISE

sired to encourage and recognize superior original research in dairy cattle nutrition and asked the American Dairy Science Association to establish rules, evaluate published work, and designate the outstanding contributions. Publications during 1946 and 1947 were eligible for the 1948 award. Four points were considered in evaluating the work, namely: original research, proper presentation, value in dairy cattle nutrition, and the possibility of practical use to the dairy cattle industry.

Nominations by many workers pointed out several leading investigations. The award committee also searched recent technical journals, proceedings, and research publications for other meritorious contributions. The selection of a series of seven publications was unanimous, based on outstanding investigations conducted at two experiment stations under the leadership of one worker. This original work scored highest. The award is for contributions on effect of prepartum diet of the cow on vitamin A and tocopherol contents of colostrum and early milk, upon vitamin storage in the newborn calf, and upon the physiology of gastric digestion in the young calf. The investigations were begun at Kansas State College and completed at Iowa State College. The man who led these investigations and receives the award is Dr. George H. Wise.

P. R. Record, vice-chairman of the Nutritional Council of the American Feed Manufacturers' Association, then presented Dr. Wise with a check for \$1,000.

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THE EFFECT OF PYRIDIUM, PENICILLIN, FURACIN, AND PHENOXETHOL UPON THE LIVABILITY OF SPERMATOOZA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN^{1, 2}

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The same types of organisms associated with vaginal infections and abortion in cows sometimes are found in the semen of bulls. A majority of the sulfonamides tested on semen samples (7) markedly reduced bacterial growth at levels which also increased the livability of the spermatozoa. Whether or not this improvement in spermatozoan livability which accompanied bacteriological control was a phenomenon distinctly associated with the sulfonamide compounds was not established.

The bacteriostatic and/or bactericidal effects of pyridium (8), penicillin (2), furacin (4, 5), and phenoxethol (3, 9) have been demonstrated on a wide variety of organisms. Almquist *et al.* (1) reported that penicillin controlled bacterial growth in diluted bull semen at levels which had no significant effect on livability or fertility. Phillips and Lardy (11) noted that "certain antibiotics, including penicillin, were not harmful to sperm motility". So far as is known to the authors, no studies have been reported on the effects of adding pyridium, furacin, and phenoxethol to diluted bull semen. The present paper is a report of the comparative effect of pyridium, furacin, phenoxethol, two penicillins, and sulfanilamide on bacterial growth and spermatozoan livability.

EXPERIMENTAL

The design and methods used in conducting the experiments reported in this paper were the same as those described previously (7). Solutions

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¹ The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, 1947.

² The authors are indebted to Merck and Company, Inc., for the pyridium; to the Eaton Laboratories, Inc., of the Norwich Pharmaceutical Company for the furacin; to the Heyden Chemical Corporation for one of the commercial penicillins, and to A. H. Allard for technical assistance in these experiments.

³ Now at the University of Illinois.

of pyridium, furacin, and phenoxethol were prepared in the same manner as were the sulfonamide solutions(7). Since penicillin comes in sterile vials and is unstable to heat, varying concentrations of penicillin solutions were prepared aseptically just prior to use in each experiment.

Eighteen first ejaculates, 13 second ejaculates, and 2 third ejaculates were used in the experiment. These had an average initial motility of 66 per cent motile spermatozoa which were moving at an average rate of 3.2, where 4.0 is considered maximum. The mean concentration was 1,180,000 spermatozoa per mm.³, and the mean methylene blue reduction time was 6.7 minutes.

Pyridium. The azo dye, pyridium, was soluble only to the extent of about 5 mg. per 100 ml. of citrate diluent. Apparently as a consequence of the low concentration, pyridium had very little effect on the livability of the spermatozoa or on the control of bacterial growth.

Five milligrams per 100 ml. of pyridium added to five semen samples stored in citrate diluent at 37.5° C. and eight semen samples stored in citrate-phosphate diluent at 20° C. had no observable effect, either beneficial or harmful, on the livability of the spermatozoa and failed to reduce bacterial growth as compared to the untreated controls.

Sulfanilamide, the positive control, was significantly superior statistically in maintaining livability of the spermatozoa. Also, it was highly effective in inhibiting bacterial growth.

Penicillin. Two commercial penicillins, *A* and *B*, each were added to four separate ejaculates stored at 20° C. in citrate-phosphate diluent at the rates of 0, 63, 125, 250, 375, and 500 Oxford Units of penicillin per ml. of diluted semen. All additions of penicillin *A* were toxic to the spermatozoa, whereas additions of penicillin *B* were not. Both penicillins were equally effective in reducing bacterial growth and were more effective than the 300 mg. per 100 ml. of sulfanilamide in this respect.

A subsequent experiment was conducted in yolk-citrate diluent at 5° C. By adding penicillin at the rate of 200, 400, 600 and 800 Oxford Units per ml. of diluted semen, penicillin *A* again was found to be toxic to the spermatozoa and penicillin *B* was not. The results are shown in table 1. As in the previous report (7), the mean motility values shown in the table were calculated from the individual observations made at specified intervals until the spermatozoa in a majority of the treatments were dead.

In maintaining the motility of the spermatozoa, sulfanilamide was found to be significantly superior, statistically, to all levels of penicillin tested. Where the treatments were not toxic, only sulfanilamide slowed the rate of motility. Whether commercial penicillin *A* was composed chiefly of a penicillin different from commercial penicillin *B* or whether it contained a substance which was toxic to the spermatozoa (12) was not determined.

In the ten samples used for the motility estimation, a study of bacterial growth after 4 and 8 days of storage revealed that bacterial contamination was small in all treatments. Neither the sulfanilamide nor the two penicillins were particularly effective in controlling the organisms which survived at 5° C. Countable plates were obtained from five of the seven ejaculates treated with penicillin B, the results of which are included in table 1. Similar results were recorded for penicillin A.

Furacin. It quickly was established that large quantities of furacin added to spermatozoa stored in the citrate-phosphate buffer at 20° C. were deleterious to the spermatozoa. Lower levels of furacin were studied on the semen obtained from four different bulls. At the four lowest levels

TABLE 1

The effect of additions of penicillin A and B to yolk-citrate upon the livability of spermatozoa and bacterial growth

Treatment	No. of ejaculates	Storage at 5° C. (days)	Controls	Oxford units of penicillin/ml.				
			SA* 300 mg. %	0	200	400	600	800
				% motile spermatozoa				
Penicillin A	3	16	37.5	35.0	31.0	27.6	21.5	15.2
Penicillin B	7	16	42.4	39.4	40.0	39.4	39.1	36.3
				Rate of motility				
Penicillin A	3	16	1.5	1.6	1.1	0.9	0.7	0.4
Penicillin B	7	16	1.4	1.6	1.5	1.5	1.6	1.5
				Bacterial count per ml.				
Penicillin A	5	4	18,500	27,000	19,000	14,000	17,500	10,000
Penicillin B	5	8	14,000	25,000	16,000	11,500	13,000	10,000

* SA = Sulfanilamide.

of furacin studied (0, 0.5, 1.0 and 2.0 mg. per 100 ml. of diluent), the mean motilities for the experiment were 42, 41, 34 and 27 per cent, respectively, the last two means being significantly different, statistically, from the first two means. Therefore, furacin was considered to be spermicidal.

Furacin proved to be an effective bacterial antagonist at the higher concentrations, but levels which were not harmful to livability of the spermatozoa were only moderately effective in controlling bacterial growth. When furacin was added to two ejaculates at the rate of 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg. per 100 ml. of diluent, the average plate count after 72 hours of storage was 81,000,000, 24,800,000, 2,760,000, 106,000, 7,000 and 650 organisms per ml., respectively. The two controls containing 300 mg. per cent of sulfanilamide averaged 2,670,000 organisms per ml. of diluted semen.

TABLE 2

The effect of adding phenoxethol to citrate-phosphate diluent at 20° C. upon livability of spermatozoa and bacterial growth (Mean of 3 ejaculates)

Agent	Conc. (%)	% motile	Rate of motility	Bacterial Count/ml. ^a
Phenoxethol	0	52.0	2.6	44,000
	0.125	20.0	0.6	8,000
	0.25	0.0	0.0	2,000
	0.50	0.0	0.0	1,500
	1.00	0.0	0.0	900
	2.00	0.0	0.0	100
Sulfanilamide	300 mg. %	53.3	2.2	22,000

^a Platings made after 24 hr.

Phenoxethol. None of the compounds previously mentioned exerted any marked bacteriostatic effect on *Pseudomonas pyocyaneus*. The reports in the literature (3, 9) that phenoxethol was effective against this organism were substantiated in this laboratory using a pure culture of pseudomonas organisms isolated from a sample of semen.

Varying concentrations of phenoxethol were added to three semen samples and were found to be highly spermicidal. In the same study, after 24 hours of storage, subsamples were taken for plating. The data are presented in table 2. While all levels of phenoxethol tested partially inhibited bacterial growth. *Pseudomonas pyocyaneus* continued to thrive at the 0.125 and 0.25 per cent levels of this drug.

Numbers of bacteria present in fresh semen. Throughout the course of these studies sufficient data were accumulated to make possible a comparative study of the numbers of bacteria present in the first and second ejaculates collected within a few minutes of each other. The data for 47 such comparisons are summarized in table 3.

TABLE 3

A comparison of the number of organisms found in first and second ejaculates collected from bulls of high and low fertility

		Range of bacteria in thousands per ml.										
Bulls	Ejaculates	1-4	5-9	10-24	25-49	50-99	100-249	250-499	500-999	1,000-2,499	2,500-4,999	Over 5,000
		No. of ejaculates in each group										
NYABO ^a	1st	2	4	3	3	6	4	2	0	1 ^b	0	1 ^b
	2nd	4	3	3	6	4	4	0	1 ^b	1 ^b	0	0
Vitamin A deficient	1st	1	0	0	0	0	4	3	7	4	2	0
	2nd	1	0	0	0	1	3	9	4	3	0	0

^a NYABO = New York Artificial Breeders Cooperative, Inc.

^b Bulls of low fertility.

Twenty-four of the 26 bulls used by the New York Artificial Breeders Cooperative, Inc., were highly fertile during the period in which the collections were made. The other two bulls were disposed of shortly thereafter because of their low fertility records. The vitamin A-deficient bulls, from which 21 paired ejaculates were collected, were not used for breeding purposes. However, they were believed to be of relatively low fertility. These results are in agreement with Gunsalus *et al.* (10), who found more bacteria in the first than in subsequent ejaculates. Furthermore, the semen from bulls of known high fertility contained fewer organisms than semen from the other bulls studied.

DISCUSSION

The data presented in this paper show that a number of compounds, chemically unrelated to the sulfonamides, effectively controlled bacterial growth. With the exception of penicillin, it was impossible to establish drug levels which were consistent with bacterial control and at the same time were not detrimental to the spermatozoa. This is in rather sharp contrast with the previous report (7) in which nine out of 12 sulfonamides in bacteriostatic or bactericidal concentrations exerted a favorable influence upon the livability of the sperm cells. This favorable influence appears to be associated with the "slowing down" of the spermatozoa noted when certain sulfonamides were present in the diluent. These phenomena were not observed when the compounds reported herein were added to the different diluents.

Penicillin, while exerting no apparent beneficial effect on the spermatozoa, was slightly superior to sulfanilamide in controlling bacterial growth. However, the decided differences in toxicity observed between the two penicillins studied present a practical problem should it become desirable to take advantage of the bactericidal properties of penicillin. The use of synthetic penicillins (6) which recently have become available offers a possible approach to the problem.

SUMMARY AND CONCLUSIONS

1. The possible usefulness of pyridium was limited by its low solubility. Maximum attainable concentrations produced no noticeable effects on spermatozoan livability or on bacterial growth.
2. Two commercial penicillins were equally effective in controlling bacterial growth and were slightly superior to sulfanilamide in this respect. Neither penicillin proved to be beneficial to the spermatozoa, and one in particular was toxic even when present in small amounts.
3. Furacin and phenoxethol proved to be highly bactericidal, and at the same time were spermicidal at most of the levels tested. Organisms of the *pseudomonas* group, especially, were resistant to the lower concentrations studied.

4. Throughout these experiments only sulfanilamide, the positive control, actually improved the livability of the spermatozoa.

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THE EFFECT OF SULFONAMIDES UPON THE LIVABILITY OF SPERMATOZOA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN¹

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The possible influence of bacteria on the results of metabolic studies and on the results of fertility in artificial insemination has been under investigation at this laboratory for some time. With the present trend of shipping the diluted semen over greater distances and inseminating animals after the diluted semen has undergone longer periods of storage, other factors, in addition to the original quality, may become increasingly important in maintaining high levels of fertility. One such factor is bacteriological control.

In view of the fact (6) that sulfonamides, with proper variation of the factors influencing sulfonamide action, inhibit the metabolism of cells of nearly every variety, and that certain sulfonamides increased the livability (8, 12) and fertility (13) of bovine spermatozoa, several sulfonamides were selected for study. Furthermore, contrary to earlier findings (3, 7, 10, 17), several investigators (5, 15, 18) reported that sulfonamides administered in human therapy did not impair the livability of the spermatozoa. Likewise *in vivo* and *in vitro* studies with rams (1) and rodents (4, 9, 11, 14) failed to demonstrate that the sulfonamides were deleterious to spermatogenesis or to the survival of the spermatozoa. Since levels of sulfonamides consistent with bacteriological control and optimum for the survival of the sperm cells had been ascertained for sulfanilamide only (8), it was logical that investigations were undertaken to study various sulfonamides in these respects.

EXPERIMENTAL

Materials and methods. Because of the large number of sulfonamides tested and the limited quantity of semen in each ejaculate, it was impossible to compare the sulfonamides with each other by adding all of them to the same series of ejaculates. Therefore, each experiment included a positive control containing sulfanilamide at the established optimum level of 300 mg. of sulfanilamide per 100 ml. of diluted semen (8), and a negative control

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¹ The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, 1947.

² Now at the University of Illinois.

containing no sulfonamide. By means of these positive and negative controls it was possible to compare the relative effects of each treatment employed.

Since length of life in storage was considered the best measure of the effect of each sulfonamide on livability of the spermatozoa, microscopic examinations to determine the percentage of motile spermatozoa and the rate of motility were made until the spermatozoa in a majority of the treatments were dead. The motility values recorded at each observation were used in calculating the mean motility values presented in the accompanying tables. Tubes containing the stored samples were coded, and at the time of each microscopic examination they were randomized to prevent the introduction of bias on the part of the investigator due to knowledge of the treatment in the particular sample under observation.

Three series of experiments were conducted. In the series I experiments, bacterial growth was measured by changes in optical density of the stored semen samples as described by Knodt and Salisbury (8) and by the plate count method. Thereafter, only the plate count method was used, for which veal infusion agar served as the culture medium. Plates were counted after 48 and 96 hours of incubation at 37.5° C. Only the total counts recorded at 96 hours are presented in this paper, inasmuch as colonies of the slow-growing diphtheroids frequently were too small to count at 48 hours.

The sulfonamides, or sulfas as they sometimes are called, selected for use were sulfathiazole (ST), sulfapyridine (SP), sulfamerazine (SM), sulfaguanidine (SG), sulfaquinoxaline (SQX), sodium sulfadiazine (NaSD), sodium sulfathiazole (NaST), carboxysulfathiazole (Carb.ST), sodium sulfamerazine (NaSM), sodium sulfamethazine (NaSMT), N¹-benzoylsulfanilamide (N¹-BSA), sulfasuxidine (SS), and sulfanilamide (SA) as a control.

All sulfonamide solutions were prepared by adding the desired quantities of each sulfonamide to the basic diluter used. These solutions then were autoclaved simultaneously at 15 lb. pressure for 20 minutes.

The 23 first ejaculates, 11 second ejaculates, and 2 third ejaculates used had a mean initial motility of 66 per cent motile spermatozoa moving at a rate of 3.3, where 4.0 is considered maximum, a mean concentration of 1,069,000 spermatozoa per mm.³, and a mean methylene blue reduction time of 6.9 minutes.

RESULTS

Series I. Semen diluted with citrate-sulfonamide diluter and stored at 37.5° C. In this series of experiments the basic diluter consisted of 3.6 g. of sodium citrate dihydrate made up to 100 ml. of water distilled in glass. One part of fresh semen was added to nine parts of the citrate-sul-

fonamide diluter and stored at 37.5° C. Six sulfonamides, at 0, 50, and 100 per cent of the maximum solubility of each drug as established for this diluter, were studied initially. Motility and optical density observations were made at 0, 6, 12, 24, and 48 hours of storage, and subsamples were taken for bacterial counts at 24 and 48 hours of storage.

After five ejaculates had been so treated, this study was terminated, for in nearly all cases most of the spermatozoa were dead after less than 12 hours of storage. Because of the adverse effect at 37.5° C. of the diluting medium upon the livability of the spermatozoa, it was impossible to measure any effect that the particular sulfonamides tested may have had on the livability of the spermatozoa.

The optical density of the stored samples increased appreciably. How-

TABLE 1

Bacterial growth in diluted semen stored with different sulfonamides at 37.5° C. as measured by plate counts
(Mean of 3 ejaculates)

Drug	Conc. (mg. %)	Rank at 24 hr.	Plate counts (1,000's/ml.) after storage for:		Rank at 48 hr.
			24 hr.	48 hr.	
SA	300	1	252	18,300	
ST	200	2	401	13,000	1
ST	100	3	9,170	26,200	3
SA	150	4	10,300	68,500	5
SM	90	5	20,200	64,000	4
SG	90	6	34,400	144,000	8
SQX	50	7	40,600	139,000	7
SP	100	8	50,700	97,000	6
SQX	25	9	66,800	178,000	9
SG	45	10	72,800	323,000	12
SM	45	11	80,100	197,000	10
SP	50	12	102,000	221,000	11
O	.	13	793,000	4,970,000	13

ever, the formation of the sulfonamide crystals in several of the treatments as the acidity increased in the stored samples interfered with the measurement of optical density changes associated with bacterial growth. Consequently only bacterial growth as measured by the plate count method will be reported. These data are summarized in table 1. The drugs are ranked on the basis of the effectiveness with which each controlled bacterial growth. Sulfanilamide, as well as several other sulfonamides, exerted a pronounced bacteriostatic effect. As a rule, bacterial growth was inversely proportional to the amount of the sulfonamide present, irrespective of the particular one used.

Series II. Semen diluted with citrate-phosphate-sulfonamide diluter and stored at 20° C. The poor livability encountered in the experiments

reported in series I was circumvented by reducing the temperature to 20° C. and by developing a more suitable diluter. The new diluter, C-P, consisting of 0.2 g. potassium dihydrogen phosphate, 0.29 g. anhydrous disodium phosphate, and 1.8 g. of sodium citrate dihydrate made up to 100 ml. water distilled in glass, was found to preserve the motility of spermatozoa for several days at 20° C. The pH of this diluent, following autoclaving, was

TABLE 2
The effect of sulfonamide additions to C-P diluter upon the percentage of motile spermatozoa
(Means for the entire experiment)

No. of ejaculates	Name of drug	Maximum sol. of each drug	% motile spermatozoa			Controls	
			Levels of the drugs expressed as % of maximum solubility				
			50	100		SA 300	mg. %
		(mg. %)					
3	ST	200	37.7	43.3	24.4	37.0	45.0
5	ST		27.4	33.2	Toxic	29.8	33.0
8	ST			36.6	Toxic	33.7	35.8
3	SP	100	37.7	32.0	27.7	37.0	45.0
5	SP		29.0	23.6	Toxic	29.8	33.0
8	SP			27.9	Toxic	33.7	35.8
3	SM	90	44.1	40.7	37.8	37.0	45.0
5	SM		32.0	28.7	N.T. ^a	29.8	33.0
8	SM			32.1	N.T.	33.7	35.8
3	SG	90	44.7	40.7	37.8	37.0	45.0
5	SG		29.2	31.0	N.T.	29.8	33.0
8	SG			34.2	N.T.	33.7	35.8
3	SQX	50	40.7	40.1	42.7	37.0	45.0
5	SQX		29.7	31.4	N.T.	29.8	33.0
8	SQX			34.4	N.T.	33.7	35.8
9	NaSD	250	34.4	34.6	31.2	30.8	33.3
3	NaST	500	17.1	13.4	9.1	23.1	22.3
4	NaST		34.0	34.0	Toxic	37.5	37.8
6	NaST			24.9	Toxic	31.2	31.3
4	Carb.ST	600 ^b	28.8	31.8	27.1	27.3	27.0
4	NaSM	500		29.8	26.8	Crystals	27.3
5	NaSMT	500		31.8	36.2	Crystals	30.6
4	N ¹ -BSA	500 ^b		26.0	22.8	5.6	26.8
4	SS	600 ^b		25.0	25.8	19.8	27.3

^a N.T. = not tested as previous tests indicated toxicity.

^b arbitrary maximum as these drugs were highly soluble.

6.90 as compared to the autoclaved citrate used previously which had a pH of about 7.75.

Motility observations were made on the semen samples at 8, 24 and 48 hours, and every 24 hours of storage thereafter, until most of the spermatozoa were dead. Usually progressive movement was observed in the diluted semen samples for at least 6 days under these conditions. In other respects, these experiments were conducted in the same manner as were those in series I.

The percentage of motile spermatozoa and the rate of motility were observed on the stored ejaculates from 15 bulls. Since it was found that with two exceptions the relative rates of motility paralleled the relative percentages of motile spermatozoa, "rate" observations will be discussed only briefly. Means for the percentage of motile spermatozoa are shown in table 2.

At the higher concentrations (100 per cent of maximum solubilities of each drug), all of the more soluble sulfonamides were toxic. When a particular level of a sulfa was established as toxic or at least not beneficial to the spermatozoa, lower concentrations of the drug were studied. These

TABLE 3

Bacterial growth in diluted semen stored with different sulfonamides at 20° C. as measured by plate counts

(Mean of 4 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc.	Rank	Plate counts (1,000's/ml.)
	(mg. %)		
ST	100	1	36 ^a
SA	300	2	52
SA	150	3	99
ST	50	4	163
NaSD	500 ^b	5	200
NaSD	250 ^b	6	252
SP	50	7	300
SP	25	8	309
SM	45	9	313
SM	22.5	10	334
SQX	25	11	417
SQX	12.5	12	547
SG	45	13	570
SG	22.5	14	1,637
O		15	5,900

^a Some colonies hidden by spreading organisms.

^b Sulfa crystals observed.

results are included in the table. Nine of the 12 drugs tested were superior to the negative controls in preserving the life of the spermatozoa, while three of the 12 drugs equaled or excelled sulfanilamide in this respect. Of these three—sodium sulfadiazine, sodium sulfamethazine, and carboxysulfathiazole—only the latter two were significantly superior to sulfanilamide (significant, respectively, at the 5.0 and 1.0 per cent levels of probability). The apparent superiority of sodium sulfamethazine and carboxysulfathiazole over sulfanilamide may be due to the fact that for some unknown reason sulfanilamide in the control failed to benefit the spermatozoa.

The additions of sulfanilamide consistently decreased the rate of movement of the spermatozoa. While the difference between the negative and positive controls was not great, it was significant statistically. N¹-benzoyl-

sulfanilamide markedly reduced the rate of movement and almost completely immobilized the spermatozoa long before they died. No particular pattern was established by the other sulfonamides.

Plate counts were made on all semen samples stored for the motility observations, but several of the samples contained large numbers of spreading organisms which prevented accurate counting. The number of bacteria in the stored semen samples from which countable plates were obtained is reported in tables 3 and 4. As was expected, the organisms

TABLE 4

Bacterial growth in diluted semen stored with different sulfonamides at 20° C. as measured by plate counts

(Mean of 2 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc.	Rank	Plate counts (1,000's/ml.)
	(mg. %)		
N1-BSA	500	1	13
SA	300	2	15
NaST	250	3	20
N1-BSA	250	4	31
NaST	500	5	33
Carb.ST	150	6	38
N1-BSA	125	6	38
NaST	125	8	40
NaSMT	500*	9	46
NaSMT	125	10	51
NaSM	500*	11	58
NaSM	250	12	64
SS	300	13	81
NaSMT	250	14	97
Carb.ST	300.	15	103
NaSM	125	16	114
Carb.ST	75	17	169
SS	75	18	176
SS	150	19	255
O	...	20	303

* Sulfa crystals observed.

multiplied rapidly at the storage temperature of 20° C. in the negative controls, although much less rapidly than at 37.5° C. Here again several sulfonamides markedly inhibited bacterial growth. Increasing the concentrations of the sodium salts of several sulfonamides beyond the point where crystallization occurred did not increase materially the bacteriostatic effect of the drugs. In one experiment, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 g., respectively, of NaSD per 100 ml. were added to diluted semen. Additions beyond the saturation point (0.25 g.) did not increase the effectiveness with which bacteria were controlled.

Series III. Semen diluted with yolk-citrate-sulfonamide diluter and stored at 5° C. Under routine conditions in artificial insemination, buffered egg yolk is used as the diluent, and the diluted semen is stored at 5° C.

Therefore, it was desirable to test, under these conditions, concentrations of the drugs which reduced bacterial growth and/or improved spermatozoan livability in the citrate-phosphate diluter at 20° C. Sulfathiazole, NaSD, NaSMT, and Carb.ST were shown to be equal or superior to sulfanilamide in at least one of these respects.

To accommodate the four drugs tested at four different levels of each drug plus a positive and negative control for each drug, five semen samples were split into 24 equal subsamples. One part of semen was added to nine parts of diluter. Motility examinations were made every 2 days until a majority of the treatments showed no motile spermatozoa. The mean

TABLE 5

The effect of ST, NaSD, NaSMT, and Carb.ST upon the livability of spermatozoa in yolk-citrate stored at 5° C.

(Mean of 5 ejaculates, and a total of 1200 observations)

Drug tested		Controls					
		No sulfa			SA 300 mg. %		
ST	mg. % in diluter	50	75	100	125	.	
	% motile sperm.	36.4	35.3	33.9	30.6	35.0	37.6
NaSD	mg. % in diluter	150	200	250	300		
	% motile sperm.	33.7	32.4	33.0	31.9	35.8	37.3
NaSMT	mg. % in diluter	100	150	200	250	.	
	% motile sperm.	33.1	33.4	29.9	26.9	35.6	38.0
Carb.ST	mg. % in diluter		100	150	200		
	% motile sperm.	39.0	40.4	36.6	31.8	36.1	38.3

motility for each of the treatments is presented in table 5. Appropriate statistical analyses revealed that the differences observed between the negative control and both carboxysulfathiazole and sulfanilamide were significant at the 5 per cent level of probability.

More than 1,000 plate counts^a were made after 4 and 8 days of storage to determine the number of organisms present in the diluted semen used for the motility observations. Most of the organisms in the five ejaculates studied did not live well at the storage temperature of 5° C. Consequently, little differentiation due to treatment was observed. At 4 days the counts

^a By washing the eggs according to Bryant and Sharp (2), wearing sterilized rubber gloves, and using all sterilized equipment, it was possible to obtain the egg yolk used in the diluent free, or almost completely free, from bacteria.

ranged from 11,000 to 43,000 organisms per ml. and at 8 days from 13,800 to 109,000 organisms per ml., with the low count being obtained from a semen sample to which sulfanilamide had been added and the high count from a sample to which no bacterial inhibitor had been added. Standard laboratory tests for the identification of *Pseudomonas pyocyaneus* showed a small percentage of pseudomonas organisms in the fresh semen of three out of the five ejaculates. After 4 and 8 days of storage, the pseudomonas organisms made up a large proportion of the total number of organisms present.

DISCUSSION

The experimental evidence presented in this paper demonstrates that all of the 12 sulfonamide preparations investigated were effective in reducing the rate of bacterial growth. Plate counts made on diluted semen stored for 24 hours at 20 and at 37.5° C. showed that sulfanilamide, sulfathiazole, and some of the other sulfonamides generally prevented the bacterial population from increasing to more than about four times that at zero time. The bacterial population increased 800 times over the population at zero time in diluted semen containing no sulfonamide.

At 5° C. and in the presence of egg yolk, none of the sulfonamides tested markedly decreased the growth of the organisms which were able to survive the storage temperature of 5° C. A majority of these surviving organisms were of the pseudomonas group. At the temperatures studied, the pseudomonas group was not inhibited markedly by levels of the sulfonamides which were not harmful to the spermatozoa.

The evidence presented shows that large doses of the sulfonamides may be toxic to the spermatozoa. However, with three exceptions, levels were established for each sulfonamide which improved the livability of the spermatozoa and, at the same time, were consistent with bacteriological control. Even at 20° C., satisfactory motility of the spermatozoa was maintained for many days in the presence of several of the sulfonamides.

While sulfanilamide fairly consistently increased the duration of motility, it consistently decreased the rate of motility. In view of the report of Knodt and Salisbury (8) that oxygen consumption by spermatozoa is depressed in the presence of sulfanilamide, this slowing down of the spermatozoa is believed to result from a depressing effect by sulfanilamide on cellular metabolism.

SUMMARY AND CONCLUSIONS

1. Twelve sulfonamides were added to bull semen diluted with citrate-phosphate and stored at 20° C. At the level of each drug determined to be optimum for spermatozoan survival, nine of the 12 drugs increased the livability of the spermatozoa over that observed when no sulfonamide was

added to the diluent. Of these nine, only two sulfonamides, sodium sulfamethazine and carboxysulfathiazole, were significantly superior to sulfanilamide in maintaining motility of the sperm cells, but they were inferior in controlling bacterial growth. Sulfanilamide slightly decreased the rate of motility, and N¹-benzoylsulfanilamide exerted a similar but more pronounced effect. No consistent pattern was established by the other sulfonamides.

2. At 20 and at 37.5° C. all of the sulfonamides were effective in reducing bacterial growth at levels which were not harmful to the spermatozoa. *Pseudomonas pyocyaneus* was not inhibited at these levels.

3. At 5° C. the sulfonamides were only slightly effective in controlling bacterial growth because the pseudomonas group of organisms predominated in the bacterial flora surviving at this temperature.

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BREEDING BEHAVIOR, SPERMATOGENESIS, AND SEMEN PRODUCTION OF MATURE DAIRY BULLS FED RATIONS LOW IN CAROTENE

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The present emphasis on extending the use of the desirably proved dairy sire in artificial breeding has focused attention on the need for more information concerning the nutrition of the mature sire, particularly with respect to the role of nutrition in semen production and fertility. In both, spermatogenesis is of primary concern.

The importance of vitamin A and carotene in developing and maintaining the normal germinal epithelium and the breeding ability of young bulls has been demonstrated by Sutton *et al.* (14), Jones *et al.* (7), Hodgson *et al.* (6) and Erb *et al.* (4, 5). Regeneration of the germinal epithelium following vitamin A and carotene therapy has been observed (4, 6). Similar studies with mature bulls have not been reported.

Roughages are the principal source of carotene for bulls of breeding age. If allowed pasture, mature bulls might be expected to build up body stores of vitamin A sufficient to carry them through periods of relatively low carotene intakes. On the other hand, the continued use of low-carotene roughages might deplete body stores to the extent that breeding ability and spermatogenic activity would be impaired. This possibility raises the question as to how long mature bulls will continue to produce normal semen and remain free from clinical manifestations of vitamin A deficiency when fed a low-carotene ration of concentrates and poor quality roughage. An investigation was undertaken in August, 1945, to measure the changes in semen production and to study the development of clinical manifestations of vitamin A deficiency in mature dairy bulls.

EXPERIMENTAL PROCEDURE

The experimental plan was to feed six relatively mature breeding bulls a low-carotene concentrate mixture along with poor quality roughage for a period of at least 1 year, unless serious clinical symptoms developed earlier. No deficiencies were apparent by the end of that period of time. Therefore, the roughage component of the ration was changed to dried beet pulp,

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and three of the bulls were fed a supplement of carotene in oil, sufficient to furnish approximately 30 mg. of carotene per 100 lb. of body weight per day. The remaining three bulls received no supplement of carotene. The 30-mg. level of carotene supplementation represented approximately 5.5 times the level for maintenance of beef bulls of equivalent weight as recommended by the Committee on Animal Nutrition of the National Research Council (11).

It was not possible at the beginning of the experiment in August, 1945, to secure six bulls that were over 5 years of age. However, semen had been collected from the two youngest bulls at irregular intervals for approximately 1 year before the initiation of the experiment. The breed and age of the bulls at the beginning of the experiment were as follows: *N*, a Holstein, age 4 years, 7 months; *C*, a Brown Swiss, age 2 years, 10 months; *J*, a Guernsey, age 8 years, 6 months; *M*, a Guernsey, age 6 years, 4 months;

TABLE 1
Carotene content of the roughages fed

Periods during which a particular roughage was fed	Kind of roughage	Carotene content (mg./lb.)
1 to 6, incl.	Hay	4.34
7 to 10, incl.	Hay	1.27
11	Hay	1.70
12	Wheat straw	1.70
13	Wheat and oat straw	0.56
14 to 16, incl.	Oat straw	1.80
17 to 21, incl.	Dried beet pulp	*

* Not analyzed; assumed to have no carotene or vitamin A potency.

K, a Guernsey, age 8 years, 7 months; *I*, a Guernsey, age 2 years, 9 months.

The feed allowance for individual bulls was established on the basis of their individual initial body weights, their appetites and their general tendency to lose or gain in body weight. Changes in the feeding schedule were infrequent. Roughage feeding was scheduled to supply approximately 60 per cent of the T.D.N. and the concentrate mixture the remaining 40 per cent.

The concentrate mixture fed consisted of 40 lb. of ground oats, 10 lb. of ground barley, 12 lb. of ground wheat, 17 lb. of wheat bran, 8 lb. of soybean oil meal, 4 lb. of linseed oil meal, 7 lb. of cane molasses, 1 lb. of steamed bone meal and 1 lb. of common salt. This mixture analyzed 0.22 mg. of carotene per pound. The calculated digestible protein content was 13.8 per cent and the T.D.N. content 71.8 per cent.

The carotene content of the roughages fed and the daily feed intakes of the bulls are shown in tables 1 and 2, respectively.

The response criteria and the methods for their measurement were as follows:

Body weights. In addition to initial and final weights on each bull, monthly average weights were secured representing one, two or three daily weights, all of which were taken within a 7-day interval, usually at the beginning of the monthly period.

Blood plasma carotene and vitamin A. The carotene and vitamin A content of blood plasma from each bull was determined by the procedure of Kimble (8) on samples obtained on 3 successive days each month.

Semen production. From each bull two ejaculates were collected at 10-day intervals by means of an artificial vagina. The volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and the per cent abnormal spermatozoa in each ejaculate were measured according to the standard procedures of this laboratory (10, 13, 15).

TABLE 2
Daily feed intakes of the bulls by periods

Periods	Kind of feed	lb. per day fed each bull					
		Bull <i>N</i>	Bull <i>C</i>	Bull <i>J</i>	Bull <i>M</i>	Bull <i>K</i>	Bull <i>I</i>
1 to 7, incl.	Concentrates	8	8	8	8	8	8
	Hay	18	18	18	18	18	12
8 to 16, incl.	Concentrates	8	8	8	7	8	8
	Hay or straw	18	18	18	14	18	12
17 to 21, incl.	Concentrates	8.4	8.2	6.2	6.2 ^a	7.2	6.2
	Dried beet pulp	12.4	12.0	9.2	9.2	10.8	9.2
	Carotene ^b (mg.)	(576)	(0)	(404)	(0)	(476)	(0)

^a During period 21, the scheduled amounts for bull *M* were reduced one-half.

^b Carotene in oil, analyzing 35,371 γ per g. of oil, was fed daily to furnish the intakes indicated. Values in parentheses are mg. per bull per day.

Clinical manifestations of vitamin A deficiency. The time intervals, in days, between the beginning of the feeding of the dried beet pulp, with and without the carotene-in-oil supplement, and the onset of night blindness, papillary edema, incoordination, edema of the extremities, loss of libido and breeding ability were noted.

Histological examination of testes. Testis tissue, obtained at the time the bulls were slaughtered, was fixed in Allen's PFA₃ fluid (modified Bowin's) (9), within 30 to 40 minutes after death. Microscopic examination of the seminiferous tubules for the extent of degeneration of the germinal epithelium was made on 7-μ sections which had been stained with an iodine-ripened hematoxylin (2) and counterstained with eosin.

Liver carotene and vitamin A. The whole livers were removed at death, stored at 40° F. and ground and sampled within 12 hours after death. A 10- to 25-g. composite sample was used for the extraction and determination of carotene and vitamin A according to the method of Davies (3).

Feed analyses. Chemical analyses of samples of the concentrates and roughages fed were made according to the methods of the A.O.A.C. (1). Carotene in the feeds as fed was estimated by the method of Nelson *et al.* (12).

The carotene content of the "carotene in oil" was determined colorimetrically by dissolving the oil in petroleum ether and measuring the density of the yellow color in a Lumitron colorimeter with a 440 m μ filter, the colorimeter previously having been standardized against pure β -carotene.

Analysis of data. In order to summarize the voluminous observations made during the experiment, the data were consolidated into twenty-one 30-day periods, each period representing three 10-day semen collection intervals. Within each of the 30-day periods, the observations made of each of the following criteria—body weights, blood plasma carotene and vitamin A, volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and per cent abnormal spermatozoa per ejaculate—were summed and the average obtained. These averages then were considered as single observations in all subsequent analyses of the data. All semen data averages were based on the combined first and second ejaculates. All bulls finished the first 16 periods but during the following 5 periods one pair of bulls, *K* and *I*, was slaughtered because of the development of severe avitaminosis A by bull *I*.

RESULTS AND DISCUSSION

The 30-day period averages for body weight, estimated daily intakes of carotene, and plasma carotene and vitamin A of each bull are presented in tables 3 and 4. During the first 16 periods (480 days), the bulls generally increased in body weight (table 3). During the final 5 periods (150 days) two of the bulls, *M* and *I*, which were not receiving the carotene supplement, showed slight losses in body weight with the onset of avitaminosis A. In contrast, bull *C* gained in weight during these latter periods.

In table 4 the plasma carotene and vitamin A values reveal some apparent deviations from a smooth curve, but, in general, these variations can be explained by the carotene intakes. The plasma vitamin A level was low (2 to 3 γ per 100 ml. plasma) for bulls *C* and *M* before pronounced deficiency symptoms were evident.

The averages for each bull, by 30-day periods, for the criteria of semen production and spermatozoan characteristics are shown in tables 5 and 6. No consistent changes in relative semen volume were demonstrated during the first 16 months of the experiment (table 5). The per cent of motile spermatozoa (table 6) showed marked decreases for bulls *C* and *I* during the 11th, 12th, and 13th periods. These decreases in motility were accompanied by equally marked increases in the per cent of abnormal spermatozoa (table 5). These concurrent changes suggest that both bulls may have been ap-

TABLE 3
Average body weights and estimated daily intakes of carotene

30-day period	Body weight in lb.						Mg. carotene fed per day					
	Bulls						Bulls					
	N	J	K	C	M	I	N	J	K	C	M	I
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture											
1	1776	1246	1535	1551	1323	1083	78.1	78.1	78.1	78.1	61.0	52.7
2	1824	1269	1553	1609	1354	1135	78.1	78.1	78.1	78.1	61.0	52.7
3	1854	1281	1541	1628	1369	1188	78.1	78.1	78.1	78.1	61.0	52.7
4	1887	1337	1581	1682	1362	1211	78.1	78.1	78.1	78.1	61.0	52.7
5	1872	1350	1584	1663	1379	1224	78.1	78.1	78.1	78.1	61.0	52.7
6							78.1	78.1	78.1	78.1	61.0	52.7
7							24.7	24.7	24.7	24.7	19.4	17.0
8	1866	1338	1603	1723	1307	1257	24.7	24.7	24.7	24.7	19.4	17.0
9	1847	1298	1536	1690	1256	1229	24.7	24.7	24.7	24.7	19.4	17.0
10	1801	1263	1495	1628	1198	1199	24.7	24.7	24.7	24.7	19.4	17.0
11	1792	1249	1422	1578	1167	1213	32.4	32.4	32.4	32.4	25.4	22.2
12	1832	1265	1423	1624	1195	1241	32.5	32.5	32.5	32.5	25.5	22.3
13							11.9	11.9	11.9	11.9	9.5	8.5
14	1903	1328	1522	1715	1247	1308	34.3	34.3	34.3	34.3	26.9	23.5
15	1897	1315	1555	1751	1289	1363	34.3	34.3	34.3	34	26.9	23.5
16	1924	1348	1588	1836	1346	1369	34.3	34.3	34.3	34.3	26.9	23.5
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K											
17	1822	1265	1520	1732	1252	1357	578	405	478	1.8	1.4	1.4
18	1825	1291	1527	1760	1269	1343	578	405	478	1.8	1.4	1.4
19	1875	1300	1485	1840	1268	1287	578	405	Slaugh-tered	1.8	1.4	Slaugh-tered
20	1958	1306	Slaugh-tered	1885	1296	Slaugh-tered	578	405	2-27-47	1.8	1.4	2-27-47
21	2024	1326		1927	1253		578	405		1.8	1.4	

* no observations made.

TABLE 4
Average micrograms carotene and vitamin A per 100 ml. blood plasma

30-day period	γ carotene per 100 ml. plasma										γ vitamin A per 100 ml. plasma									
	Bulls					Bulls					Bulls					Bulls				
	N	J	K	C	M	I	N	J	K	C	M	I	N	J	K	C	M	I	N	J
Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture																				
Aug. '45	114	58	105	43	79	82	21	15	10	12	9	12	21	15	10	33	24	27	12	12
1	50	52	95	42	69	70	24	17	14	16	12	13	12	17	14	24	15	16	13	13
2	38	57	81	42	70	82	17	14	9	14	14	12	14	14	9	16	12	15	12	12
3	39	59	91	41	71	94	24	21	13	21	17	16	24	21	13	21	17	16	16	16
4	34	51	99	41	69	82	17	15	13	16	15	12	17	15	13	16	15	12	12	12
5
6	50	73	142	60	108	162	28	24	22	33	24	27	28	24	22	33	24	27	27	27
7	41	72	127	52	89	110	17	16	14	24	15	16	17	16	14	24	15	16	16	16
8	25	54	82	35	60	77	15	16	14	18	12	15	15	16	14	18	12	15	15	15
9	28	49	62	29	53	61	17	16	13	16	10	13	17	16	13	16	10	13	13	13
10	28	44	60	28	53	69	14	17	15	15	13	16	14	17	15	15	13	16	16	16
11	45	110	139	53	133	109	29	30	31	28	22	23	29	30	31	28	22	23	23	23
12	36	68	123	41	97	92	22	22	18	22	17	19	22	22	18	22	17	19	19	19
13	40	71	104	47	99	94	14	12	9	12	9	9	14	12	9	12	9	9	9	9
14	45	71	118	50	100	97	23	24	18	25	16	18	23	24	18	25	16	18	18	18
15	57	82	115	63	132	128	25	25	22	29	22	22	25	25	22	29	22	22	22	22
16	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K																			
Dec. '46	144	192	244	42	60	70	27	28	22	15	9	11	27	28	22	15	9	11	11	11
17	155	197	374	14	13	25	28	26	23	6	5	6	28	26	23	6	5	6	6	6
18	126	199	231 ^b	14	19	20	24	23	6 ^b	4	3	5	24	23	6 ^b	4	3	5	5	5
19	308	400	Slaughtered	13	18	Slaughtered	37	32	Slaughtered	3	3	Slaughtered	37	32	Slaughtered	3	3	Slaughtered	3	3
20	198	163	2-27-47	8	15	2-27-47	37	22	2-27-47	3	2	2-27-47	37	22	2-27-47	3	2	2-27-47	2-27-47	2-27-47
21	a. no observations made.																			

a. no observations made.

b. Bloated and off feed resulting in a decreased carotene intake and probably, therefore, a lowered plasma carotene and vitamin A.

proaching a deficiency state. In the case of bull *I*, the average number of spermatozoa per milliliter of semen (table 5) decreased during these same periods.

During the last 5 months, when bulls *C*, *M* and *I* were on the beet pulp and low-carotene concentrate mixture without the carotene supplement, semen volume again was characterized by considerable variation without a definite trend accompanying the low plasma vitamin A values and the onset of clinical symptoms. At the same time, the per cent of motile spermatozoa decreased and the per cent of abnormal spermatozoa increased as these bulls developed advanced vitamin A deficiency (table 6).

The concentration of spermatozoa per milliliter of semen for the combined first and second ejaculates varied considerably up to the time the last samples were collected. Of those bulls receiving no carotene supplement bull *I* exhibited an increase in spermatozoan concentration while bulls *C* and *M* exhibited decreases in concentration just prior to the time they were unable to mount.

The bulls receiving the carotene supplement exhibited sufficient variation in the several semen and spermatozoan characteristics that no particular improvement was attributed to the increased carotene intake.

Figure 1 shows photomicrographs of histological sections of the seminiferous tubules of the right testis of each bull. These photomicrographs are by pairs of bulls and show the contrasting conditions of the tubules accompanying the presence and absence of supplemental carotene in the basal ration. Bull *C* exhibited the most advanced degeneration of the germinal epithelium, followed by bull *I* and bull *M* in order. All exhibited a similar pattern of degeneration, namely, few spermatogonia, relatively small numbers of spermatocytes and immature spermatids. The seminiferous tubules of the bulls receiving the carotene supplement apparently were normal, with the exception of bull *K*, and showed numerous dividing spermatogonia, spermatocytes, maturing spermatids, and many spermatozoa in the lumen of the tubules.

It should be pointed out that the testes sections of bull *K* showed occasional tubules which were characterized by almost complete degeneration of the germinal epithelium and the absence of spermatids and spermatozoa in the lumen. Whether or not these represented unrepaired tubules arising from the feeding regime prior to the carotene supplementation is, in the absence of biopsy data, mere speculation.

Table 7 presents for comparison the observations on the onset of the clinical symptoms in terms of days after the change in roughage from hay and straw to beet pulp for those bulls not receiving the carotene supplement. Considerable variation occurred between bulls in the rapidity with which they developed incoordination, edema of the extremities and night blindness. This variation, undoubtedly, was a reflection of their respective

TABLE 5

Average semen volume per ejaculate and numbers of spermatozoa per ml.
(Averages represent first and second ejaculates)

30-day period	Semen volume per ejaculation in ml.						Numbers of spermatozoa per ml. $\times 10^6$					
	Bulls						Bulls					
	N	J	K	C	M	I	N	J	K	C	M	I
Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture												
Aug. '45	4.3	5.6	3.9	4.1	6.3	3.8	1.21	1.23	0.60	1.09	1.16	0.89
1	6.4	5.5	6.5	4.3	5.9	3.3	1.04	1.17	0.92	0.96	1.08	0.82
2	4.4	4.2	5.8	4.3	6.8	3.1	1.45	1.11	0.78	1.54	1.13	1.05
3	4.7	5.8	7.2	3.9	8.3	2.6	1.48	1.20	0.77	1.39	1.06	0.80
4	4.5	5.2	6.6	3.5	6.9	2.5	0.99	0.99	0.76	0.81	1.31	0.81
5	3.5	6.1	7.0	5.5	6.2	2.2	1.11	1.28	0.59	1.18	1.15	0.81
6	4.3	4.6	6.0	4.6	6.6	2.5	0.79	1.14	0.59	0.88	1.12	0.61
7	3.6	4.7	5.2	5.9	6.3	2.7	1.12	0.76	0.69	1.13	0.86	0.64
8	3.9	4.4	4.4	4.8	5.5	2.1	1.21	0.90	0.52	0.74	1.08	0.49
9	3.5	3.8	5.5	4.2	5.2	3.0	0.90	1.17	1.10	0.98	0.83	0.48
10	3.6	5.4	a	5.4	4.3	1.6	1.04	1.12	"	1.40	1.06	0.38
11	2.9	2.7	a	4.0	7.1	3.4	0.92	0.80	"	1.08	1.28	0.50
12	2.7	6.2	4.6	4.1	5.9	3.4	0.71	1.16	0.62	0.97	0.68	0.64
13	3.3	4.8	2.9	4.9	5.8	3.0	0.80	0.84	0.52	1.07	0.83	0.64
14	2.9	5.5	7.5	4.6	5.9	2.8	1.00	0.94	0.74	0.77	0.93	0.44
15	4.2	5.4	8.0	4.5	7.7	3.0	0.79	1.12	0.56	1.03	1.04	0.71
Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K												
Dec. '46	4.0	6.6	6.0	4.5	6.2	3.6	0.89	0.73	0.86	0.70	1.05	0.56
17	5.9	6.9	7.1	5.6	5.7	3.7	1.28	0.76	0.90	0.97	1.27	0.97
18	6.8	6.8	6.5c	5.5	6.1	b	1.22	1.14	0.64c	0.79	1.22	b
19	6.0	7.9	Slaughtered	5.6	5.0	Slaughtered	1.17	0.87	Slaughtered	0.61	1.07	Slaughtered
20	7.4	6.7	2-27-47	3.9	3.1	2-27-47	1.14	0.83	2-27-47	0.21	0.73	2-27-47

a No semen collected.

b Unable to mount.

c Represents only one collection during the period.

TABLE 6
Average percentages of motile and of morphologically abnormal spermatozoa
(Averages represent first and second ejaculates)

30-day period	% motile spermatozoa												% morphologically abnormal spermatozoa											
	Bulls						Bulls						Bulls											
	N	J	K	C	M	I	N	J	K	C	M	I	N	J	K	C	M	I						
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture																							
1	58	58	68	62	59	63	5	12	13	26	15							21						
2	61	53	53	56	54	43	11	10	10	20	19							22						
3	55	53	43	39	59	42	9	13	11	17	16							32						
4	59	65	67	67	56	61	9	17	14	22	21							40						
5	62	58	48	55	48	56	11	13	16	12	20							29						
6	60	60	51	53	55	53	16	13	17	15	19							33						
7	65	63	73	63	64	59	12	14	16	15	18							32						
8	53	56	55	57	62	58	9	14	15	15	19							30						
9	66	59	52	62	60	60	11	11	15	10	19							29						
10	66	54	60	50	53	44	12	13	15	11	22							47						
11	66	63	a	19	61	33	10	10	a	66	20							85						
12	65	57	a	38	54	34	8	12	a	27	22							55						
13	62	48	49	47	58	49	9	16	13	19	17							25						
14	53	50	43	58	41	52	10	15	16	7	18							30						
15	53	53	55	53	50	44	9	14	10	6	16							27						
16	50	46	45	53	43	47	7	12	12	10	15							24						
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K																							
17	60	48	45	57	51	31	7	9	9	8	11							25						
18	65	47	53	44	44	29	6	8	9	13	12							24						
19	52	46	30 ^c	40	44	b	6	8	8 ^c	20	13							b						
20	56	53	Slaugh- tered	36	36	Slaugh- tered	4	9	Slaugh- tered	31	26							Slaugh- tered						
21	67	45	2-27-47	45	20	2-27-47	3	12	2-27-47	64	68							2-27-47						

^a No semen collected.

^b Unable to mount.

^c Represents only one collection during the period.

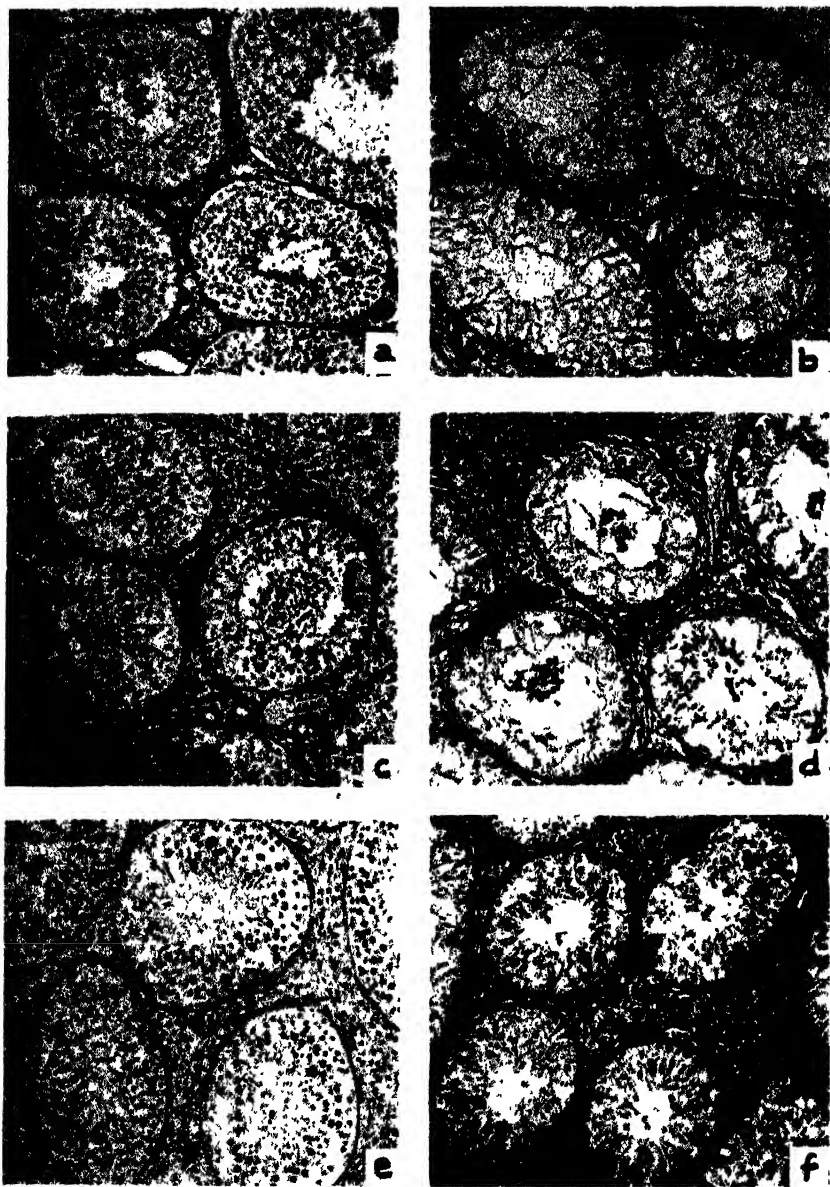


FIG. 1. Photomicrographs of seminiferous tubules of the right testis of each bull at time of slaughter. ($\times 100$.) a, c and e—bulls *N*, *K* and *J* after 151, 73 and 151 days, respectively, on the ration supplemented with carotene. b, d and f—bulls *C*, *I* and *M* after 151, 73 and 151 days, respectively, on the ration unsupplemented with carotene.

TABLE 7

Number of days bulls were fed deficient ration before onset of first clinical symptoms of vitamin A deficiency

Bull	Off feed	First signs of incoordination	Unable to mount	Extreme incoordination	Stiffness in legs	Marked edema of knees, hocks, and ankles	Night blindness ^a
C	^b	90	140	140	130-140	150	150 ^c
M	38 ^d	120	150 ^e	150	150	^f	150 ^g
I	38	40	50-60	70	60	70	^f

^a All bulls on the unsupplemented ration showed varying degrees of characteristic bleaching of the tapetum lucidum.

^b Bull C was never off feed.

^c Hemorrhagic areas about the papilla of right eye.

^d Bull M was off and on feed several times during the course of the feeding of the deficient diet. Scouring was also a frequent accompaniment of his erratic appetite.

^e Bull M was sufficiently incoordinated that he undoubtedly would not have been able to mount in another 10 days.

^f Condition not in evidence at completion of experiment.

^g It was questionable whether this bull was actually night blind.

body stores of carotene and vitamin A and their rates of depletion on the experimental rations employed.

In general, it would appear that incoordination and loss of the ability to mount, without the loss of libido, are the earliest signs of vitamin A deficiency in mature breeding bulls and occur before any marked impairment in semen production is manifested.

The liver stores of carotene and vitamin A at the time of slaughter are shown in table 8. The values reflect clearly the rations fed and the breeds used.

From the evidence of this investigation and the reports of others, the

TABLE 8

Carotene and vitamin A content of the fresh livers at the time of completion of experiment for individual bulls

Bull	Breed	No. of days between date of change of ration and date of slaughter	γ per g. fresh liver	
			Carotene	Vitamin A
Ration: Dried beet pulp, low carotene concentrate supplement of carotene-in-oil			mixture, plus daily	
N	Holstien	151	438	778
J	Guernsey	151	1335	273
K	Guernsey	73	873	73
Ration: Dried beet pulp and low-carotene concentrate mixture				
C	Brown Swiss	151	28	12
M	Guernsey	151	48	13
I	Guernsey	73	47	5

authors believe that, while typical clinical manifestations of vitamin A deficiency and degeneration of the germinal epithelium can be produced in mature dairy bulls, the feeding of poor quality dry roughages, presumably low in carotene, for extended periods of time will not likely cause noticeable impairment of semen production before the onset of the clinical manifestations of A deficiency.

SUMMARY AND CONCLUSIONS

Six dairy bulls were fed dry roughages low in carotene and a concentrate deficient in carotene and vitamin A for a period of 16 months without inducing clinical manifestations of vitamin A deficiency or noticeable impairment of semen production.

Subsequent changes in the roughage component of the ration of three of the bulls from hay and/or straw to dried beet pulp plus the same concentrate mixture resulted in the development of incoordination, edema of the extremities, papillary hemorrhage, a gradual increase in the per cent of abnormal spermatozoa and a decrease in the per cent of motile spermatozoa, but no consistent change in the number of spermatozoa per milliliter of semen within a period of 40 to 120 days. These three bulls were unable to mount but still retained an unusual amount of libido. This inability to mount was manifested before the changes in semen production.

Typical patterns of degeneration of the germinal epithelium of the seminiferous tubules were found in the three bulls on the carotene-deficient rations. There were few spermatogonia, spermatocytes, spermatids or maturing spermatozoa in the lumen of the tubules.

While supplementing the carotene-deficient ration of the other three bulls with carotene in oil prevented the degeneration of the germinal epithelium which characterized those bulls not receiving the supplement of carotene in oil, it produced no consistent changes in semen production which reasonably could be attributed to the carotene.

Liver carotene and vitamin A levels of the carotene-deficient bulls were of the order of 30 to 50 γ and 5 to 13 γ per g. of fresh liver, respectively.

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FURTHER STUDIES OF THE NUTRITIVE VALUE OF BUTTERFAT FRACTIONS¹

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It was reported (4) that butterfat collected in September, 1945, was fractionated by crystallization from acetone at -4° C. and yielded two fractions; a liquid one (II) which promoted growth in rats at a superior rate as compared with butterfat or corn oil, and a solid one (I) which allowed a significantly inferior growth rate. This phenomenon was not observed to the same degree in later trials when samples of butter collected in subsequent months, including September, 1946, were used. Since the pasture of the summer of 1947 was considered as generally better than that of 1946, it was thought advisable to repeat the same fractionation procedure using the late summer butterfat from cows on this better pasture.

Fractionation of milk fat by cold crystallization also has been reported by Jack *et al.* In their early work (5) they did not obtain any one fraction with a growth promoting action superior to that of the whole milk fat. In a recent paper (6), they report that by the use of purified solvents they obtained a milk fat fraction which produced a significantly better growth in young rats than the original milk fat and also greater growth than supported by the other milk fat fractions. They attributed their first results to the effect of impurities in the solvents on the fat.

EXPERIMENTAL

The September, 1947, butter obtained from the University dairy was fractionated in the same manner described in the first paper of this series (1), again using purified acetone as the solvent, and at a temperature of -5° C. Fraction I had a melting point of 42° C., and an iodine number of 21, fraction II, a melting point of 7° C., and an iodine number of 51.

The fractions, as well as the untreated butterfat and corn oil, were used as the separate sources of fat in the diets fed to four groups of six rats each. Male weanling rats of the Sprague-Dawley strain weighing 40–50 g. were placed on the diets, the basic composition of which is shown in table 1.

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² Government of India Research Fellow.

TABLE 1
Composition of the diets

Components	28% Diet	10% Diet
Fat ^a	28	10
Sucrose	48	66
Casein ^b	20	20
Salts IV ^c	4	4
Vitamin supplement mg. per 100 g. diet		
Thiamine		0.2
Riboflavin		0.3
Pyridoxine		0.3
Ca pantothenate		1.5
Choline hydrochloride		150
β -Carotene ^d		0.56
α -Tocopherol		2.24
Calciferol ^e		
2-Methol-1,4-naphthoquinone		0.21

^a Butterfat or corn oil or one of the fractions.

^b Extracted for three 2-hour periods with boiling 95% alcohol.

^c Phillips, P. H., and Hart, E. B., J. Biol. Chem., 109: 657. 1936.

^d 90% β -carotene and 10% α -carotene.

^e Crystalline irradiated ergosterol.

The fat level of the diet in this experiment was 28 per cent. The rats were housed in individual metal cages with raised screen bottoms, watered and fed daily *ad libitum* and weighed once weekly. Consumption records were kept during the fifth week, but since the growth of the groups on the corn oil and fraction I diets was irregular during this week, only the efficiency of the butterfat and fraction II diets was calculated.

Table 2 gives the gain in weight of the rats for 5- and 6-week periods. It also includes the efficiency of each of the two diets mentioned above in terms of grams of diet consumed per gram of weight gained.

Even though the differences in growth in this experiment were not as wide as those obtained in 1945, fraction II again showed a striking superiority over fraction I, and over the butterfat and corn oil. Furthermore, the efficiency of the diet containing this fraction (II) was found higher than that containing whole butterfat. On comparing the gains on the butterfat itself to those on the corn oil, some superiority of the former is apparent with these sucrose diets.

TABLE 2
Growth gains and efficiency values on 28 per cent fat diets

	Butterfat	Corn Oil	Fraction I	Fraction II
	g.	g.	g.	g.
5-week gain	170	165	149	186
6-week gain	190	184	168	217
Grams diet/gram gain during 5th week	2.5			2.22

In repeating this experiment for further verification, it was decided to replace the corn oil diet by another containing Crisco (a partially hydrogenated cottonseed oil) as the source of fat. This was done in view of the reports by Kentie (7) and Boer *et al.* (2), (3), who claimed that the superiority of summer butter is due to its content of vaccenic acid and also because it was possible by Bertram's method (1) to isolate from Crisco a substance presumably corresponding to vaccenic acid. Also, the entire series was repeated on diets containing 10 per cent fat (table 1) in order to investigate the possibility that while a growth-promoting factor is being concentrated in fraction II, a retarding growth factor might be left in fraction I, and when fed at a 28 per cent level, would inhibit growth. Table 3 represents the various diets used and the corresponding weight gains during a 5-week period. During the sixth week, some of the rats were killed for microbiological analysis of their cecal flora.

TABLE 3

Growth gains during the 5-week period on 28 and 10 per cent fat diets

	Butterfat	Crisco	Fraction I	Fraction II
28% fat	143	144	.	157
10% fat	146	137	130	161

In comparing the values of the first experiment in table 2 to those displayed in table 3, generally poorer growth of the latter series becomes obvious. This is an unfortunate phenomenon in this type of study, and one which emphasizes the need for large numbers of experimental animals. However, within this series, the superiority of fraction II over the butterfat itself again is indicated both on the 28 and the 10 per cent level. The Crisco, from which have been isolated large amounts of the substance presumably corresponding to vaccenic acid, did not stimulate better growth than did butterfat.

In this experiment no significant difference seemed to have resulted from the varying levels of fat in the diets. However, work in progress points to the probability of the presence in certain fats of an inhibiting factor which exerts its effect to a noticeable degree when the fat represents a higher portion of the diet.

SUMMARY

September butterfat (1947) again was fractionated by crystallization from acetone at -5°C ., yielding a liquid fraction (II) which allowed rats to grow at a superior rate as compared with butterfat or corn oil, and a solid fraction (I) which gave a significantly slower growth rate.

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SACRAL DEFORMITY IN THE "WRYTAIL" ABNORMALITY IN CATTLE¹

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The importance and the possible mode of inheritance of "wrytail" in cattle have been presented by Atkeson and Warren (2) and by Atkeson *et al.* (1). These workers point out that the "wrytail" character has been observed in the Guernsey, Holstein-Friesian, Ayrshire, Brown Swiss and Jersey breeds. Their data indicate its inheritance as a single autosomal recessive. Observations of the present authors have extended to four breeds heretofore unreported and include 137 cattle of different ages and both sexes, as follows: Beef Shorthorns, 51; Aberdeen Angus, 39; Hereford, 17; Red Polled, 30. The only case of wrytail found was in one Red Polled cow in which the tailhead was set to the left.

A "wrytail", according to Atkeson *et al.* (1) is a malformation consisting of a distortion of the tail head, the base of the tail being set at an angle to the back bone instead of in line with it. The "tailhead" is the term commonly used by dairy cattle workers and judges to designate the area limited by the first three coccygeal vertebrae. The "wrytail" malformation therefore, would seem to be at or near the junction of the fifth sacral and first coccygeal vertebrae.

Roemmele (5) described a condition in Brown Allgauer cattle as being somewhat similar to "wrytail". Essentially, this malformation involved a twisting of the coccygeal vertebrae at a point posterior to the tail head. While the malformation described by Roemmele is similar in the effect on the vertebrae and intervertebral discs, the region affected makes it more nearly resemble "screwtail" (Knapp *et al.* (4)) than "wrytail".

EXPERIMENTAL

The present investigation involves a study of the anatomical features of the "wrytail" condition in a 7-year-old purebred Jersey female. Inasmuch as this was a case of definite wrytail and no similar analysis is known to have been attempted, it is thought the results may be of interest to others. In this case the tail was set to the right as indicated in figure 1. This condition was first observed when she was a 2-year-old. It is not

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known how long the character was present or if it was present at birth, as prior observations with this in mind had not been made. Three months after this picture was taken, the cow was slaughtered and a section of the rump, approximating 60 lb., was removed. This portion included the sacrum, most of the tail, and parts of the ilia. From the tissues thus involved, that portion of the tail and sacrum shown in figure 2 was prepared by removing the soft tissue, first by boiling water and, subsequently, by removing soft tissue with a scalpel and, finally, with steel wool. The sacrum was

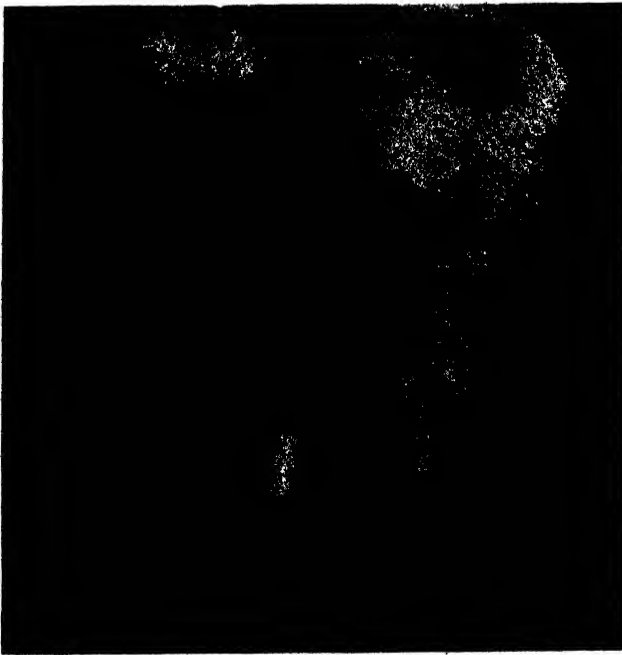


FIG. 1. Dorsal aspect of sacro-coccygeal region on a 7-year-old multiparous Jersey cow, no. 264.

then compared with the normal as described by Ellenberger and Baum (3) and by Sisson and Grossman (6). No evidence of trauma or inflammatory change (in terms of callus formation, thickening, or exostosis) was discovered.

The sacrum in this subject was 25 cm. in over-all length, 19 cm. in width anteriorly (alae) and 7.8 cm. in width at the extreme posterior extremity. Sacral segments I-IV, inclusive, were fused in their bodies, and in their spinous, transverse, and articular processes. The over-all length represented by fused segments I-IV, inclusive, was 20 cm. The fifth sacral segment was not fused at the junction of its body or transverse,

spinous, or articular processes with those of the fourth segment. The fifth dorsal and ventral sacral foramina thus were incomplete and that part of the lateral sacral crest, contributed to by the articular processes of sacral segments IV and V, also was incomplete. A lateral declination of approximately 12° to the right from the longitudinal axis common to sacral segments I-III, inclusive, to the axis common to sacral segments IV and V was noted. No declination was noted between the longitudinal axis of



FIG. 2. Ventral aspect of sacrum and first three coccygeal vertebrae of Jersey cow no. 264, showing sacral deformity.

sacral segment V and that possessed in common by the first three coccygeal vertebrae (fig. 3).

Three radiographs were taken of this area, one before death and two after death. The one taken during life (11-15-46) demonstrated that the deformity did not lie in the anterior coccygeal region. The second (fig 3), taken of the frozen rump, revealed the site of the deformity to be in the sacrum. The final radiograph (fig. 4) was made of the sacro-coccygeal re-

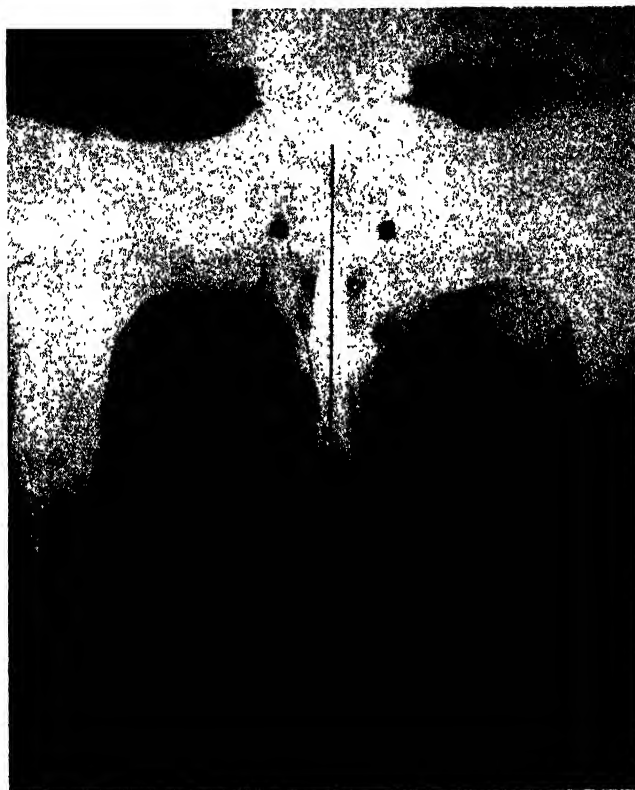


FIG. 3. Radiograph of sacro-coccygeal region, in section of rump removed from carcass at slaughter and then frozen. Dorsal view. Cow no. 264.

gion after removing the soft tissue. The specimen was laid flat on the cassette (*i.e.*, with the sacral alae depending over the edge) and a series of exposure made of the area apparently involved in the deformity (segments III-V, inclusive). The exposures were made on six portions of one large

TABLE 1
Detailed radiographic study of sacral segments III-V, inclusive

Exposure no.	Voltage	Distance	Tube current reading	Time
	(kilovolts)	(in.)	(milliamps.)	(sec.)
1	60	30	52	0.1
2	60	30	52	0.25
3	60	30	52	0.5
4	40	30	55	0.25
5	40	30	55	0.5
6	40	30	55	0.75

film, using lead plates to delineate the areas. Time and intensity variations were introduced, as noted in table 1.

The first series (exposures 1-3, inclusive) showed greater delineation of articulation or fusion (as the case might be) of adjacent segments, and midline detail in general. The second series (exposures 4-6, inclusive) showed greater delineation of transverse process structure.

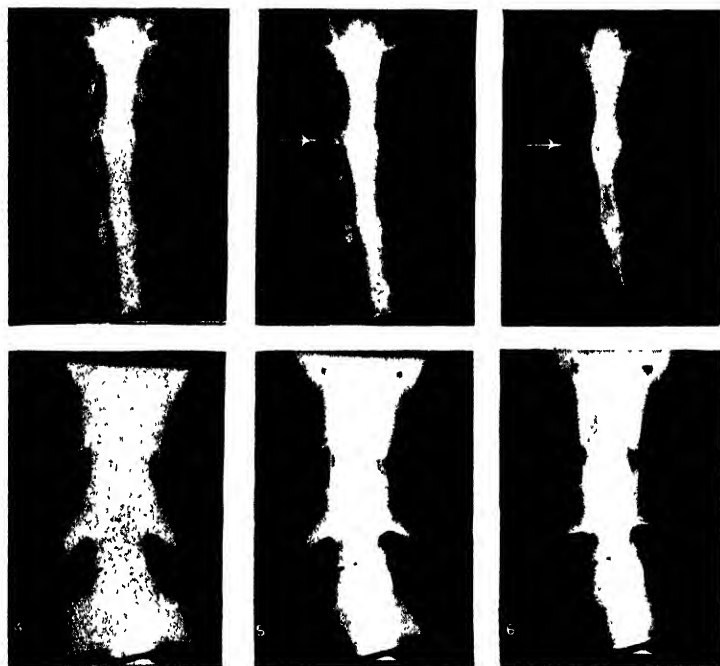


FIG. 4. Detailed radiographic study of sacral segments III-IV, Dorsal view (see table 1). Arrow indicates wedge-shaped area of fusion between segments III-IV. Cow no. 264.

An over-all lateral declination (right) of about 12° was noted, occurring at the fusion point of segments III-IV (7°) and within segment IV itself (5°). Since the specimen lay flat on the cassette at the time of exposure, and since the anode-film distance was relatively great and the perpendicularity of the central ray to the film was carefully checked, it was felt that linear and angular measurements made from the film would be valid. A measurement of declination of involved segments, made from the film (exposure 3, fig. 4), showed approximately 7° lateral declination (right) of the longitudinal axis (perpendicular to the line of fusion) of the anterior portion of sacral segment IV, from the longitudinal axis of segment III. An additional lateral declination of approximately 5°

(right) of the longitudinal axis of the posterior portion of segment IV (also perpendicular to the transverse axis of the articulation concerned, i.e., IV-V) from the longitudinal axis of the anterior portion, was noted.

On exposure 3, figure 4, a measurement of the intersegmental distance at the fusion point demonstrated a slight wedge-shape of that area. At its central and right portions, this area measured approximately 2 mm. in antero-posterior thickness. At its visibly expanded left extremity, it measured 3 mm. in antero-posterior thickness. The right lateral portion was not as amenable to accurate measurement on the X-ray film as the other portions of the fusion area, due to the partial obscurity cast by the spinous process. However, the wedge-shape and general measurements of this area were verified on the specimen itself. External measurements on the specimen are given in table 2.

Measurements also were made on right and left sagittal longitudinal axes of segment IV, at a distance of 12 mm. to either side of the ventral

TABLE 2

External measurements of intersegmental fusion areas of sacrum

Articulation (fusion area) of sacral segments	Transverse diameter of articulation ^a	Ventral intersegmental distance, antero-posterior		
		Left, 12 mm. from midpoint	Mid- point	Right, 12 mm. from midpoint
	(mm.)	(mm.)	(mm.)	(mm.)
I-II	39	5.0	2.0	5.0
II-III	35	3.5	2.75	3.25
III-IV	28	3.0	2.5	2.0

^a Calipers and dividers were used in making measurements.

midline (the distance 12 mm. was chosen arbitrarily, since differences seemed fairly pronounced and more easily measurable at that distance). The length of the right sagittal axis was found to be 47 mm. as compared to 50 mm. for the length of the corresponding left sagittal axis.

CONCLUSIONS

Examination of the sacral and coccygeal vertebrae of one animal showed the "wrytail" malformation to involve the sacrum rather than the tail-head in the case studied.

The extent and direction of the malformation as measured by the angle of declination was 12° right.

From radiographic and other observations, it appears that one locus of bone growth disparity lay in the fusion area between sacral segments III and IV. An additional declination (42 per cent of the total) occurred within the body of sacral segment IV.

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FROZEN HOMOGENIZED MILK. IV. KEEPING QUALITY OF FROZEN HOMOGENIZED MILK AFTER THAWING

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Previous studies (2, 5, 6, 7, 9) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Later studies (3, 4, 8) showed that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample. However, changes in the temperature at which frozen homogenized milk was stored did not affect materially the chemical composition of the different sections of quart samples. The concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen.

The literature does not contain information regarding the keeping quality of homogenized milk after it has been frozen and then thawed. The present study therefore was undertaken to determine the effect that storage of frozen homogenized milk has on its keeping quality after thawing.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. This milk had been pasteurized at 155° F. for 30 minutes.

Samples of the fresh homogenized milk immediately were stored at 30.5° C., 15.5° C., 12.8° C., 7.22° C., and 1.67° C. Other samples were frozen immediately and held at -27.5° C. for various periods, after which they were thawed and then stored at the same temperatures as those at which the fresh milk samples had been held. At regular intervals a one-half pint sample of milk was removed from storage in order to determine its bacterial content, titratable acidity, pH, and flavor. The first three of these determinations were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men experienced in milk judging.

RESULTS

The flavor developments in homogenized milk held for different periods of time before and after freezing are shown in table 1. This table shows

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TABLE 1
Flavor of homogenized milk before and after freezing

[illegible]

TABLE 2
Bacterial growth in homogenized milk before and after freezing

		Bacteria per millimeter									
Storage temperature	Storage period	Before freezing	No. of days frozen milk stored at -27.5° C.								
			3	17	24	31	45	66	87	111	150
(°C.)	(days)										
Fresh	...	800									
30.5	1	11,600,000						11,248,000	31,400,000	70,000,000	30,000,000
15.5	1	850	600								
	2	6,500	1,300								
	3	278,000	133,000								
	4	4,000,000	1,980,000								
Fresh	...	300									
12.8	1	400	400								
	2	400	500								
	4	800	300								
	5	5,300	300								
	6	4,800									
	7	975,000	80,000								
	8	560,000	351,000								
7.22	2	490	1,300								
	4	7,000	600	400	900						
	6	152,000	123,000	600	300	400	300				
	8	2,770,000	160,000	69,000	117,000	167,000	90,000				
	10	6,000,000	220,000	4,860,000	780,000	140,000	134,000				
	12	12,000,000	3,500,000	21,500,000	92,000,000	165,000	500,000				
1.67	2	800				310,000	1,230,000				
	4	1,000						300	1,000		1,600
	7	1,200						300	200	300	1,000
	9	1,600						300	500	500	1,900
	11	5,000						300	400	1,100	600
	14	180,000						11,200	12,000	123,000	36,000
	16							78,000	11,400	95,800	600,000
	18	960,000						160,000			13,800,000
	21	1,500,000						420,000	342,000		1,000,000
								1,000,000	780,000		

that storage has practically the same effect on the flavor of both the fresh homogenized milk and the homogenized milk that had been held in the frozen state. The samples that were stored at 30.5° C. were sour when examined at the end of 24 hours. Those which previously had been stored for 111 days and 129 days at -27.5° C. were unclean or putrid, indicating that the rate of flavor deterioration may be slightly more rapid in milk that has

TABLE 3

Titratable acidity development in homogenized milk before and after freezing
(Standard methods (1) procedure used)

Storage temperature	Storage period	Titratable acidity (% lactic acid)										
		Before freezing	No. of days frozen milk stored at -27.5° C.									
			3	17	24	31	45	66	87	111	129	150
(°C.)	(days)											
Fresh		0.12										
30.5	1	0.36						0.37	0.36	0.34	0.35	
15.5	1	0.12	0.12									
	2	0.12	0.12									
	3	0.12	0.12									
	4	0.17	0.16									
Fresh		0.12										
12.8	1	0.12	0.12									
	2	0.12	0.12									
	4	0.12	0.13									
	5	0.12	0.13									
	6	0.13	0.13									
	7	0.13	0.13									
	8	0.14	0.12									
7.22	2	0.12	0.12	0.12	0.12							
	4	0.12	0.12	0.12	0.12	0.12						
	6	0.13	0.13	0.13	0.12	0.13						
	8	0.14	0.13	0.13	0.14	0.13	0.15					
	10	0.28	0.13	0.17	0.16	0.15	0.16					
	12	0.45	0.27	0.20	0.33	0.16						
1.67	2	0.12						0.13	0.13		0.13	0.14
	4	0.12						0.12	0.13	0.12	0.15	0.14
	7	0.13							0.12	0.12		0.14
	9	0.13						0.14	0.12	0.12	0.15	0.14
	11	0.14						0.13	0.12	0.13	0.15	
	14	0.15						0.13	0.12	0.17	0.14	0.14
	16							0.15	0.13			
	18	0.18						0.18	0.15			
	21	0.20							0.16			

been held in the frozen state. However, there appeared to be no appreciable difference in the rate of flavor deterioration between the two milks when lower storage temperatures were used.

Table 1 further shows that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods of time than usually are required to hold fluid milk before use.

To determine whether bacteria multiply faster and acidity develops more rapidly in homogenized milk which has been frozen and then thawed than in the corresponding fresh homogenized milk, samples of the two milks were stored at different temperatures. The initial bacterial count, acidity, and pH values were determined prior to placing the milks in storage. After different storage periods, these determinations were repeated. The results are given in tables 2, 3 and 4.

TABLE 4
pH changes in homogenized milk before and after freezing

Storage temperature	Storage period	pH									
		Before freezing	No. of days frozen milk stored at -27.5° C.								
			3	17	24	31	45	66	87	111	129 150
(°C.)	(days)										
Fresh	"	6.73									
30.5	1	5.40						5.28	5.43	5.96	5.46
15.5	1	6.65	6.52								
	2	6.61	6.52								
	3	6.52	6.57								
	4	6.37	6.43								
Fresh	"	6.70									
12.8	1	6.69	6.18								
	2	6.61	6.65								
	4	6.33	6.51								
	5	6.70	6.43								
	6		6.46								
	7	6.60	6.45								
	8	6.44	6.47								
7.22	2	6.63	6.52	6.55	6.46						
	4	6.52	6.57	6.50	6.37	6.64	6.50				
	6	6.58	6.55		6.42	6.43	6.42				
	8	6.50	6.46	6.50	6.52	6.52	6.48				
	10	6.01	6.52	6.23	6.48	6.48	6.48				
	12	5.34	5.84	6.00	5.82	6.48					
1.67	2	6.50						6.57	6.61		6.62 6.74
	4	6.58						6.58	6.73	6.64	6.80 6.68
	7	6.66							6.62	6.62	6.48
	9	6.50						6.58	6.68	6.52	6.68 6.50
	11	6.48						6.56	6.70	6.60	6.62
	14	6.50						6.58	6.72	6.54	6.62 6.69
	16							6.52	6.62		
	18	6.40						6.49	6.52		
	21	5.90							6.48		

Table 2 shows that, as previously reported (3), freezing milk and storing it in the frozen state had a tendency to lower the number of bacteria per ml. as determined by the standard plate count. The initial bacterial counts of the fresh homogenized milk were higher than those of the corresponding homogenized milk which had been held in the frozen state. These differences, however, were not consistently reflected in the counts made after various periods of storage. This indicates that from a bacteriological standpoint there is no significant difference between fresh homogenized milk and homogenized milk which has been held in the frozen state.

Tables 3 and 4 show that acidity develops at practically the same rate in homogenized milk when thawed, after being held in the frozen state, as it does in fresh homogenized milk when both are held under similar conditions.

CONCLUSIONS

The changes in flavor during the storage of homogenized milk which has been held in the frozen state and then thawed were similar to those of fresh homogenized milk. Frozen homogenized milk of good quality may be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods than usually are required to hold fluid milk before use.

From a bacteriological standpoint, as determined by standard plate counts, there was no significant difference between homogenized milk which has been stored in the frozen state and then thawed and fresh homogenized milk.

Homogenized milk that had been stored in the frozen state and then thawed showed no significant difference from fresh homogenized milk in the development of acid as measured by titratable acidity and pH determinations.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FROZEN HOMOGENIZED MILK. V. EFFECT OF AGE BEFORE FREEZING ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

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Earlier studies (2, 6, 7, 9, 10) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Previous studies (3, 4, 8) also have shown that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample during the freezing process; apparently there was no further movement of these solids after the milk was frozen. A more recent study (5) has shown that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration for longer periods of time than usually are necessary before use.

The literature does not contain information regarding the effect of the age of homogenized milk before freezing on its keeping quality after freezing. The present study was undertaken to determine the effect of age before freezing on the keeping quality of frozen homogenized milk.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. The milk had been pasteurized at 155° F. for 30 minutes. Fifty-six samples were taken directly from the filler and divided into seven groups of eight samples each. One sample was examined immediately for flavor, bacterial count, coliform organisms, titratable acidity, pH, and sediment and the remaining seven samples of this group were placed in a freezer held at -17.5° C.² The remaining samples were held at 1.67° C. Eight samples, each representing a separate group, were removed from the 1.67° C. storage after 12, 24, 48, 72, 96 and 120 hours. Each time, one of the eight samples of the respective group was used for laboratory examination and the remaining seven were placed in a freezer at -17.5° C. The samples were removed from the -17.5° C. storage and thawed for laboratory examination after having been held in the frozen state for 5, 28, 38, 47, 56, 76, and 86 days. The determinations for bacterial content,

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² The thermostat controlling the temperature of this freezer had sufficient lag to cause a temperature variation of about eight degrees.

TABLE 1
Effect of age before freezing on the keeping quality of frozen homogenised milk

Age before freezing (Hr.)	Not frozen	No. of days frozen					Flavor	V. Sl. ox.	Sl. ox. ^a	Sl. stale—ox.	86
		5	28	38	47	56					
0	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
12	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
24	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
48	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
72	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
96	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
120	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Sl. stale—ox.	
Bacterial counts ^b											
0	3600 +	1000 +	2100 +	1400 -	1600 -	2200 -				4900 +	
12	3400 -	1000 +	1500 -	1600 -	1600 +	2600 -				2800 -	
24	1500 -	1300 -	1200 -	1300 -	1800 -	1600 -				2600 -	
48	900 -	900 -	2500 -	1100 +	1100 -	900 -				2300 -	
72	700 -	600 -	1800 +	1700 -	1000 -	1500 +				2500 -	
96	1100 -	600 -	1300 -	1200 +	1400 -	1100 -				1100 -	
120	1100 +	700 -	500 -	1100 -	800 -	1600 +				1200 -	

TABLE 1 (continued)
Effect of age before freezing on the keeping quality of frozen homogenized milk

	Sediment						Acidity					
	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	Titra- table	pH	Titra- table	pH	Titra- table	pH
0	0.02	0.02	0.25	0.40	0.60	0.60	0.65	0.60	1.20	1.20	1.20	4.50
12	0.03	0.02	0.10	0.60	0.25	0.70	1.30	3.50	1.30	1.30	1.30	3.50
24	0.03	0.02	0.10	0.40	0.30	1.10	2.20	3.80	2.20	2.20	2.20	3.80
48	0.02	0.02	0.55	0.20	0.50	0.60	2.70	2.50	2.70	2.70	2.70	2.50
72	0.02	0.03	0.30	0.90	0.60	1.10	2.60	4.50	1.10	1.10	1.10	4.50
96	0.02	0.02	0.40	0.50	1.30	1.60	2.20	2.20	1.60	1.60	1.60	2.20
120	0.02	0.02	0.20	0.40	0.65	2.00	3.00	3.00	2.00	2.00	2.00	3.00

	Acidity						Acidity					
	Titra- table	pH	Titra- table	pH	Titra- table	pH	Titra- table	pH	Titra- table	pH	Titra- table	pH
0	0.115	6.67	0.125	6.62	0.115	6.62	0.110	6.66	0.115	6.68	0.130	6.56
12	0.125	6.79	0.120	6.63	0.115	6.58	0.110	6.62	0.115	6.62	0.130	6.55
24	0.120	6.64	0.120	6.61	0.120	6.60	0.110	6.63	0.115	6.64	0.125	6.61
48	0.120	6.60	0.115	6.53	0.115	6.72	0.115	6.62	0.110	6.67	0.125	6.59
72	0.120	6.63	0.110	6.59	0.110	6.72	0.115	6.61	0.110	6.64	0.130	6.63
96	0.120	6.65	0.115	6.65-	0.115	6.58-	0.115	6.60	0.110	6.53	0.130	6.62
120	0.120	6.64	0.115	6.62	0.115	6.62	0.110	6.61	0.115	6.63	0.130	6.42

* ox. = oxidised

b + and - denote presence or absence of coliform organisms in 1-ml. samples.

coliform organisms, titratable acidity, and pH were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men, all of whom were experienced in milk judging. The sediment was determined by the method used by the authors in their earlier studies (2, 3).

RESULTS

The effect of age before freezing on the keeping quality of frozen homogenized milk is shown in table 1. The milk was of good flavor throughout the 120 hours that it was held at 1.67° C. before freezing. The flavor of the milk remained good when it was held in the frozen state for 47 days, regardless of its age before freezing. Some of the samples that were thawed after they had been frozen for 56 days and for 76 days had developed a slight oxidized flavor. However, there was no correlation between the age of the samples before freezing and the development of this flavor. When the samples were thawed after holding in the frozen state for 86 days, they had a stale and oxidized flavor. There was an insignificant tendency for these flavors to be more pronounced as the age of the samples before freezing was increased.

Table 1 also shows that there was no significant change in the bacterial content of the samples either in the 120 hours that they were held at 1.67° C. or in the 89 days that they were held in the frozen state at -17.5° C. There apparently was a slight coliform contamination of the milk that was used in the preparation of the samples. With the exception of those samples which were held at 1.67° C. for 24 hours before freezing, at least one sample in each age group was positive for coliform organisms in 1-ml. portions either before or after freezing. Two of the samples before freezing and two each of those held in the frozen state for 5, 28, 47 and 56 days gave positive coliform tests. Of these samples held in the frozen state for 47 and for 86 days, one each gave a positive test. There was no correlation between the positive coliform tests in the samples before and after freezing.

Table 1 shows further that the quantity of sediment remained low and constant in the milk samples before they were frozen. It did not increase when the samples were held in the frozen state for 5 days. When held for 38 days, however, the quantity of sediment had materially increased and it continued gradually to increase as the storage time lengthened. Significant separation, as shown by the sediment readings, had occurred when the samples were thawed after they had been held for 47 days in the frozen state, and considerable sediment was present in all the samples when they were thawed after having been frozen for 89 days. There was, how-

ever, no correlation between the degree of separation and the age of the sample prior to freezing.

The acidity as shown by titration and by pH determinations did not vary significantly either before the samples were frozen or while they were held in the frozen state. The titratable acidity ranged from 0.110 to 0.130 per cent and the pH values from 6.51 to 6.79. These variations were well within the limits of experimental error. The titratable acidity values were lower than usually encountered because of the dilution technic employed.

CONCLUSION

It is recognized that homogenized milk should be frozen as soon as possible after processing, but if the milk is of good quality it may be kept as long as 120 hours at 1.67° C. before freezing without adversely affecting the keeping quality of the frozen product.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FERTILITY LEVEL OF BULL SEMEN DILUTED AT 1:400 WITH AND WITHOUT SULFANILAMIDE

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The basic advantage of artificial insemination, the ability to breed more cows to superior sires than can be done in natural service, can be realized on a broad practical basis only if the single ejaculate of a superior bull can be divided and many cows bred with it. The practical difficulty involved in inseminating extremely small volumes of semen has resolved the problem to standardizing the volume inseminated at 1.0 ml. and diluting the semen with appropriate amounts of diluter. This was done to reduce the spermatozoa numbers to a level consistent with maximum efficiency of use of semen and optimum fertility.

Earlier studies (3, 4, 5) have shown that bull semen may be diluted at levels as high as 1 part of semen to 100 parts of the yolk-citrate diluter with no detectable effect on the fertility level. The present report deals with experiments designed to test even wider dilution rates.

EXPERIMENTAL PROCEDURE

The investigations reported here were conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc. The general methods used in handling the semen and the methods used in determining the results of each insemination have been reported earlier (3, 4, 5). Two investigations were made. The first was to study the effect of levels of dilution above 1:100 when the yolk-citrate diluter was used. This diluter was composed of equal parts fresh egg yolk and a buffer composed of 3.6 g. of sodium citrate dihydrate per 100 ml. of water distilled in glass and autoclaved for 20 minutes at 15 lb. pressure.

Preliminary evidence suggested that some decrease in fertility might be expected at dilution rates above 1:100. Therefore, a carefully designed experiment was conducted using the semen of one bull of consistently high fertility. The experiment was in the form of a 4 × 4 Latin square, each of four ejaculates being split into four aliquots and each of these aliquots being diluted at rates of one part of semen to 100, 200, 400 and 800 parts of the yolk-citrate diluter. It was impossible for each of the 75 different inseminators involved in this study to use semen diluted at each level for each of the ejaculates used. Therefore, the inseminators were divided arbitrarily into four groups. Each group received semen diluted at one

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single rate for one particular collection, but each group received all dilution rates at some time during the experiment. Such a design, although somewhat cumbersome to manipulate in the central headquarters from which the semen was shipped, presented no difficulty in the field and permitted precise measurements of the results.

The second experiment was with 19 different bulls and was designed as a randomized block experiment. Each bull was considered as a block. Five collections were made from each bull with intervals of approximately 10 days between collections. Dilution rates were assigned at random to these collections. Each semen collection was diluted at one rate, i.e., one part of semen to 100, 150, 200, 300 or 400 parts of the diluter used. The semen was shipped to all inseminators using semen of that particular breed. Four breeds were represented. The diluter used was composed of equal parts egg yolk and a sodium citrate-sulfanilamide buffer consisting of 3.6 g. sodium citrate dihydrate and 0.6 g. sulfanilamide made up to 100 ml. with water distilled in glass. The final concentration of sulfanilamide in the diluter was 300 mg. per 100 ml. This experiment, although not giving as precise estimates of the sources of random variation as the first, was much more extensive in scope, and more general conclusions and recommendations for practice could be drawn from it.

A statistical study also was made of the relation between the number of spermatozoa inseminated and fertility level in the case of approximately 700 ejaculates of semen routinely used in artificial insemination between January 1 and August 31, 1947.

RESULTS

In the first experiment the average numbers of spermatozoa in 1 ml. of diluted semen at each dilution rate were: for 1:100, 14,580,000; for 1:200, 7,330,000; for 1:400, 3,670,000 and for the 1:800 dilution rate, 1,840,000 spermatozoa per 1 ml. inseminated. The results are presented in table 1 and include services to cows bred for the first and for the second time in a service period. An analysis of covariance using services as the independent and 165-day (5-month) non-returns as the dependent variable showed that the differences observed were significant, the 1:400 and 1:800 rates being different from the 1:100 rate. Also, it should be noted that the cows bred with the semen diluted at 1:100 apparently held to service better than did the cows bred with semen diluted at the higher rates. This fact was of considerable interest and prompted the second experiment.

In the second experiment the average number of spermatozoa in 1 ml. of the diluted semen was: for 1:100, 12,060,000; 1:150, 8,490,000; 1:200, 6,840,000; 1:300, 4,160,000 and for the 1:400 dilution rate, 3,290,000 spermatozoa. The means of the semen quality characteristics are presented in table 2. Statistical analyses of these data show that none of the differ-

TABLE 1

Fertility of the semen of one bull diluted at various levels with yolk-citrate, 45, 75 and 165 days after insemination

	Ratio of semen to diluter				Total
	1: 100	1: 200	1: 400	1: 800	
Services	98	106	82	70	356
% non-returns					
Av. 45 days	76.5	63.2	56.1	61.4	64.6
Av. 75 days	67.3	50.9	45.1	47.1	53.4
Av. 165 days	61.2	48.1	36.6	44.3	48.3

ences were significant. Thus, it is concluded that the design did not bias the experiment towards different qualities of semen for use in preparation of different dilutions.

The results of the inseminations are shown in table 3. They represent only those services to cows being bred for the first time in a service period and are given as 45-day (1 month), 75-day (2 month) and 165-day (5 month) non-returns to service. Statistical analysis of the data indicated that the mean differences in fertility level were not statistically significant. In fact, the variance due to treatments from the analysis of covariance and that due to random variation in the data were almost identical, the F value being 1.01 for 75-day non-returns and 0.78 for 165-day non-returns. However, a trend toward a decrease in fertility with each decrease in the number of spermatozoa inseminated is apparent in the mean values for each dilution rate given in table 3. The correlation between the number of spermatozoa inseminated and the fertility of each ejaculate used in the experiment was 0.24, a small but significant figure at the 5 per cent level of probability. The relationship indicated by the correlation coefficient explained but a minor portion of the variance in fertility level observed. The regression of fertility level on spermatozoa numbers inseminated was linear. Between the limits of numbers of spermatozoa per insemination used, the calculated regression formula was $Y = 51.27 + 0.777X$, where Y = estimated per cent of 165-day non-returns and X = the number of spermatozoa inseminated. This

TABLE 2

Average of the semen quality characteristics for each dilution rate

	Ratio of semen to diluter				
	1: 100	1: 150	1: 200	1: 300	1: 400
Initial motility, %	72.1	71.8	71.3	71.8	71.1
Concentration, 1,000's/ mm. ³	1,218	1,282	1,274	1,253	1,318
Methylene blue reduction time, min.	5.0	4.9	4.7	4.9	4.6

result is equivalent to a change in fertility level of approximately 0.8 per cent for each change of one million spermatozoa between the limits used in the experiment.

Similar calculations for approximately 700 ejaculates used routinely in artificial insemination resulted in a smaller but a statistically significant regression coefficient. The regression equation was $Y = 56.86 + 0.3146X$ or equivalent to a change of approximately 0.3 per cent in fertility for each change of one million spermatozoa inseminated. However, the regression calculated on this latter data was for a different range of spermatozoa numbers inseminated than was the case of the experimental data. The extreme ranges of spermatozoa numbers inseminated in the routine work were from 6,700,000 to 34,600,000 per insemination, with the mean being 14,700,000 spermatozoa. In contrast, the range in the last experiment was from 2,360,000 to 15,300,000 spermatozoa per insemination.

TABLE 3

Fertility of the semen of 19 bulls diluted at various rates with yolk-citrate sulfanilamide, 45, 75 and 165 days after insemination

	Ratio of semen to diluter					Total
	1: 100	1: 150	1: 200	1: 300	1: 400	
Total services	1408	1580	1581	1379	1395	7343
% non-returns						
Av. 45 days	69.1	64.3	66.7	62.6	62.6	65.1
Av. 75 days	60.9	56.3	58.4	55.0	52.8	56.7
Av. 165 days	58.1	53.2	55.4	51.3	48.5	53.3

DISCUSSION

This series of investigations to determine the optimum dilution rate for fertile bull semen and the minimum number of bovine spermatozoa required for maintenance of optimum fertility in artificial insemination appears to present a number of problems, some of which have been answered only partially. In the first place, the data reported earlier (3, 4, 5) showed no decrease in fertility as the numbers of spermatozoa inseminated were decreased from approximately 400 million down to approximately 13 million spermatozoa per insemination. In those experiments, yolk-citrate diluter, made up of equal parts of fresh egg-yolk and a solution containing 3.6 g. or slightly more of sodium citrate dihydrate per 100 ml. of water distilled in glass was used.

When the numbers of spermatozoa were reduced further by increased dilution with the same diluter used in the first experiment reported here, the decrease in fertility noted was large and the differences were significant. This fact suggested that the minimum number of spermatozoa per insemination for optimum fertility had been reached. In contrast, however, the second experiment, in which sulfanilamide was added to the diluter, failed

to show the same great decrease in fertility level over a range of spermatozoa numbers similar to that used in the first experiment. A downward trend in fertility was suggested by the regression calculation. An even smaller downward trend in fertility with decreasing spermatozoa numbers was shown for semen used in routine artificial insemination work in which the yolk-citrate sulfanilamide diluter was regularly used.

These facts suggest that the curve of declining fertility with decreasing spermatozoa numbers probably is a logarithmic one in which the plateau of approach to the optimum is long and the slope very small. However, as the minimum is approached, the rate of decrease in fertility accelerates, the slope of the curve becomes greater and the fertility level probably reaches zero before spermatozoa numbers reach that level.

Secondly, the data presented here and other evidence from this laboratory suggest that the position of the curve, although perhaps not its slope, may be altered to some degree by the diluter in which the spermatozoa are suspended. It has been shown that the livability of bull spermatozoa is shortened by greater dilutions (4). Also, it has been shown that bull spermatozoa in low concentrations are harmed by oxygen (2). Sulfanilamide depresses the oxygen consumption by spermatozoa, stimulates increased livability (1), and improves the fertility of spermatozoa used for insemination after storage (6). It is not known that sulfanilamide will increase the inherent fertility of bull semen if that semen were used for insemination immediately after collection. Rather, the effect in increasing fertility earlier observed (6) is believed to be due to prolongation of innate fertilizing capacity rather than an actual increase in the potential.

Based on these observations it is suggested that the addition of sulfanilamide to the diluter in the last experiment reported here preserved the life of the spermatozoa in low concentrations better than was done by the yolk-citrate. Thus, the downward acceleration of the fertility level was partially prevented at the levels of spermatozoa numbers used. However, it is believed that with somewhat lower numbers of spermatozoa, the accelerated decrease in fertility would be observed. Until more fundamental information is available leading to control of the metabolic processes involved, it appears that the minimum number of spermatozoa from fertile bulls which should be used for insemination of cows rests at between 5 and 10 millions per insemination. In the case of particularly valuable proved sires that are highly fertile, lower numbers of spermatozoa can be used but a sacrifice in fertility level probably would result.

Finally, it should be emphasized that these experiments were carried out under field conditions in which shipment of semen was routine. Most inseminations were made the second, third and fourth days after the semen was collected. The semen was from normal bulls of high fertility. It was of excellent quality, as shown in table 2. However, these experiments do not

enable one to speculate on the probable fertility of highly diluted semen from bulls of low fertility. Nor do the minimum numbers mentioned above imply that bulls producing semen of low spermatozoa count will be fertile.

SUMMARY

In one investigation it was found that the practical limit of dilution rate was about 1:100 when the yolk-citrate diluter was used. In another experiment involving 7,343 inseminations when sulfanilamide was added to the yolk-citrate diluter at the rate of 300 mg. per 100 ml., no difference was found in fertility level between dilution rates of one part of semen to 100, 150, 200, 300 and 400 parts of the yolk-citrate-sulfanilamide diluter.

However, a trend downwards amounting to 0.8 per cent in fertility level for each decrease of 1 million spermatozoa inseminated was observed, over the range of 2.36 to 15.30 millions of spermatozoa inseminated. The probable reasons for the different results of the two experiments are discussed.

With present handling and insemination techniques, it is suggested that the minimum number of spermatozoa consistent with optimum fertility rests at 5 to 10 millions from bulls of known fertility.

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AN ANALYSIS OF THE RESULTS OF THE 1947 COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

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A few items on the score cards used in the Collegiate Students' International Contest in the Judging of Dairy Products frequently have been questioned as to their real values in testing the judging abilities of the contestants. The inference sometimes is made that certain items are scored as effectively by the lower ranking contestants as by the higher ranking contestants. If this is true, these items may have little weight in determining the judging abilities of contestants. Some coaches of dairy products judging teams have felt that high ranking contestants attain their standing, in part at least, because of extreme conservatism in certain phases of scoring.

The contestants' score cards from the 1947 contest were made available to determine if high standings were attained without actually showing proficiency in judging. Also, it seemed desirable to determine if any techniques were employed by the winning contestants that could be used in the training of judging teams.

PROCEDURE

The data included herein were obtained from a study of the score cards of 57 contestants. Each scored ten samples of creamery butter, milk, Cheddar cheese and vanilla ice cream. Hereafter, these products will be designated as butter, milk, cheese and ice cream. Two hundred and twenty-eight contestant cards were examined and 2,280 judgments were involved.

The contestants' score cards for butter, milk, cheese and ice cream were grouped into quartiles. The first quartile consisted of the cards of the 14 contestants scoring highest in the judging of that specific product; the second quartile contained the cards of the 14 next highest contestants, and so on. The quartiles for the different products may or may not have represented the same contestants. It should be pointed out that the fourth quartile included cards from contestants who through carelessness failed by omission or commission to score properly the various items and, therefore, were given the maximum penalty. For this reason the data obtained from the cards in the fourth quartile may not be significant.

DISCUSSION OF THE DATA

An examination of the data presented in tables 1 to 4, inclusive, reveals
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TABLE 1

The grades (points lost) of the three high individuals and quartiles of contestants in the judging of butter

Class	Points lost in scoring								Total
	Flavor		Body & texture		Color		Salt		
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	7.00	1.00	0.0	0.0	0.0	0.0	0	0	8.00
2nd “	7.00	2.50	0.0	0.0	0.0	0.0	0	0	9.50
3rd “	9.00	2.00	0.0	0.0	0.0	0.0	0	0	11.00
Av. of 3 high individuals	7.66	1.83	0.0	0.0	0.0	0.0	0	0	9.49
1st quartile	9.04	3.25	0.29	0.43	0.0	0.0	0	0	13.01
2nd “	11.39	3.93	0.96	1.43	0.0	0.0	0	0	17.71
3rd “	12.00	3.71	2.14	1.29	0.11	0.07	0	0	19.32
4th “	16.43	5.20	3.67	3.67	0.0	0.0	0	0	28.97

information very similar to that noted in previous studies (1, 2); i.e., the high ranking contestants in each product attained that position because they knew how to score properly the "flavor" and "body and texture."

The following deductions from the data may be helpful:

Flavor. The three high contestants in the scoring of the specific products were not significantly better than the others in criticizing the flavor of cheese, ice cream and milk. However, the three high individuals showed superior ability to describe and evaluate the flavor of butter. Generally all contestants lost more points in placing a score on flavor than they did in recognizing a flavor.

Body and texture. In cheese judging, the two high individuals surpassed their close competitors both in recognizing defects of body and tex-

TABLE 2

The grades (points lost) of the three high individuals and quartiles in the judging of Cheddar cheese

Class	Points lost in scoring						Total
	Flavor		Body & texture		Color		
	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	9.00	6.00	4.50	1.50	1.00	2.00	24.00
2nd "	9.00	5.50	4.00	3.00	1.50	3.00	26.00
3rd "	10.50	6.50	7.00	3.50	1.00	2.00	30.50
Av. of 3 high individuals	9.50	6.00	5.16	2.66	1.16	2.33	26.81
1st quartile	11.18	6.14	7.39	4.04	1.14	2.14	32.03
2nd "	13.89	7.18	7.86	5.79	1.39	2.57	38.68
3rd "	15.04	7.00	11.64	5.96	1.18	2.36	43.18
4th "	20.10	7.57	12.50	6.60	2.10 ^a	3.10	51.97

^a Only 13 contestants involved. Data from two contestants were so abnormally out of line they were not included.

TABLE 3

The grades (points lost) of the three high individuals and quartiles in the judging of milk

Class	Points lost in scoring					Total
	Flavor		Sediment		Container & closure	
	Score	Criticism	Score	Score	Criticism	
1st individual	8.50	6.00	3.00	0.50	0.50	18.50
2nd "	18.00	4.00	2.25	0.25	1.00	25.50
3rd "	12.00	6.50	2.50	2.25	3.50	26.75
Av. of 3 high individuals	12.83	5.50	2.58	1.00	1.66	23.57
1st quartile	16.50	5.62	4.08	2.09	1.99	30.28
2nd "	20.96	6.51	4.68	1.48	2.42	36.05
3rd "	25.14	6.35	6.17	1.96	2.99	42.61
4th "	30.30	7.08	8.11	2.55	3.03	51.07

ture and in weighing the criticism. In body and texture of butter, the first three individuals agreed precisely with the judge and the contestants in the first quartile did not vary too widely from the judge. However, in criticizing ice cream, the three high contestants were not significantly better than those in the first quartile; in evaluating the criticism, the two highest individuals were somewhat superior to their immediate competitors.

Miscellaneous items. Neither the judges nor the contestants criticized the salt content in butter in this contest. However, some contestants criticized the color of butter, but the judge did not do so.

In the judging of the color of cheese, the three high individuals did no better than did those in the first three quartiles.

Practically all contestants scored and criticized similarly the melting quality of ice cream. However, the scoring of color of ice cream was evidently a "booby trap" for some of the contestants, especially those in the fourth quartile.

TABLE 4

The grades (points lost) of the three high individuals and quartiles in the judging of vanilla ice cream

Class	Points lost in scoring								Final grade
	Flavor		Body & texture		Melting quality		Color		
	Score	Criti- cism	Score	Criti- cism	Score	Criti- cism	Score	Criti- cism	
1st individual	12.00	5.70	3.50	2.50	2.50	3.00	0.0	0.0	29.20
2nd "	15.50	6.50	4.50	2.50	1.50	1.00	0.0	0.0	31.50
3rd "	16.00	7.50	6.00	2.00	0.50	0.00	0.0	0.0	32.00
Av. of 3 high individuals	14.50	6.57	4.66	2.33	1.50	1.33	0.0	0.0	30.89
1st quartile	16.17	6.05	5.79	2.79	1.93	2.04	0.04	0.07	34.88
2nd "	17.39	6.34	7.93	4.76	2.14	2.64	0.32	0.14	41.66
3rd "	22.04	7.32	9.38	5.26	2.29	2.36	0.29	0.29	49.23
4th "	21.57	7.52	10.50	7.33	2.23	2.30	4.30	0.33	56.08

In scoring sediment of milk, it is possible that the three high individuals retained a more accurate mental picture of the sediment standards than other contestants and thereby attained a better score. The item "container and closure" of milk evidently had been so well impressed upon the minds of all contestants that little variation existed in the scoring and criticizing of this item.

The fourth quartile. Table 5 was compiled to demonstrate in what area the contestants of the fourth quartile failed most seriously. The data were secured by subtracting the average grade of the first quartile from that of the fourth quartile. The data seem to indicate that the chief weakness of the contestants represented in the fourth quartile was their inability to place a correct score on the flavor of the product being judged. In criticizing the flavor of the products, there was little difference between contestants of the first and fourth quartiles. This is indicated by the fact

TABLE 5
Difference in scores between 1st and 4th quartiles

Product	Score card items											
	Flavor		Body & texture		Color		Sediment		Container & closure		Melting quality	
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Score	Criticism	Score	Criticism	
Butter ...	7.39	1.95	3.39	3.25								
Cheese ...	8.92	1.43	5.11	2.56	1.06	0.96						
Milk ...	13.80	1.46					4.03	0.46	1.04			
Ice cream	4.87	1.47	4.71	4.54	4.27	0.26				0.30	0.26	

that the fourth quartile lags behind only 1.95 points in butter, 1.43 points in cheese, 1.46 points in milk and 1.47 points in ice cream. A marked difference existed between the first and fourth quartiles when the sense of touch (body and texture criticisms) was involved. This is shown by the divergence of the scores of body and texture in butter, cheese and ice cream. Contestants of the fourth quartile lost as many points in scoring body and texture of ice cream as they did in scoring flavor. Likewise, they lost heavily in the scoring of body and texture in cheese. The ratio between the criticism of body and texture and its score is narrow. From the data available it is not possible to make a precise observation on this point. The solution to the difficulty may lie in devoting more time to the development of the sense of touch.

Effect of range of score on contestant rating. It has been alleged that contestants, when in doubt as to the correct score, tend to judge an item conservatively. Official judges sometimes are criticized similarly. Data in table 6 indicate that official judges used about 75, 57, 63 and 89 per cent of

TABLE 6

Range in scores of officials and contestants in scoring flavor of dairy products

Class	Range of score used in scoring flavor of							
	Butter (8) ^a		Cheese (7) ^a		Milk (15) ^a		Ice cream (9) ^a	
	Range	%	Range	%	Range	%	Range	%
Official	6.0	75.0	4.0	57.1	9.5	63.3	8.0	88.9
1st individual . . .	5.0	62.5	4.0	57.1	15.0	100.0	3.5	38.9
2nd "	5.0	62.5	4.5	64.3	6.5	43.3	3.5	38.9
3rd "	5.0	62.5	5.0	71.4	10.0	66.7	3.0	33.3
1st quartile	5.5	68.8	4.5	64.3	9.9	66.0	4.4	48.9
2nd "	5.2	65.0	3.8	54.3	9.6	64.0	4.2	46.7
3rd "	5.2	65.0	3.6	51.4	8.0	53.3	5.0	55.5
4th "	5.3	66.3	5.3	75.7	9.4	62.7	4.7	52.2

^a Normal range in flavor score for the product.

the normal range in scoring the flavor of butter, cheese, milk and ice cream, respectively.

In the scoring of butter for flavor, contestants of the first quartile used a slightly greater percentage of the normal range than did contestants of the remaining quartiles. The three highest individuals used a very slightly lower proportion of the normal range than did the average of the four quartiles.

The official judge of cheese was more conservative in the use of the score range of flavor than the judges of the other products. The ranking individual used the same range. Contestants making up the second and third quartiles were even more conservative. On the other hand, individuals composing the fourth quartile used about 76 per cent of the recommended range.

TABLE 7

Range in scores of officials and contestants in scoring body and texture of cheese and ice cream

Class	Range of score used in scoring body and texture of			
	Cheese (3.5) ^a		Ice cream (4.5) ^a	
	Actual range	% of normal range	Actual range	% of normal range
Official	3.5	100.0	2.0	44.4
1st individual	2.0	57.1	1.5	33.3
2nd "	2.5	71.4	1.5	33.3
3rd "	3.0	85.7	1.0	22.2
1st quartile	2.2	62.8	1.8	40.0
2nd "	2.3	65.7	1.6	35.5
3rd "	1.8	51.4	1.6	35.5
4th "	2.4	68.5	1.5	33.3

^a Normal range in body and texture score for the product.

In scoring milk for flavor, most of the contestants used about the same range as the official judge. The winning contestant used the entire normal range. This contestant was not outstanding in determining the flavor defects, but he did a superior job in evaluating the flavor defect. The contestant winning second place in the scoring of milk used less than half the percentage of the normal range used by the first place winner, yet this contestant lost 10.5 more points in evaluating flavor than did the individual placing first.

All students, regardless of quartile or individual standing, seemed to be unusually moderate in the scoring of the flavor of ice cream. The official judge utilized approximately 89 per cent of the normal range. Most of the contestants used one-third to one-half of the recommended range.

Contestants were more conservative than the official judge in using the recommended range for body and texture of cheese (table 7). The official judge of cheese used 100 per cent of the normal range. The official judge and all of the contestants were extremely conservative in using the recommended range of body and texture of ice cream.

GENERAL DISCUSSION

The score cards as set up for the judging of butter, cheese, milk and ice cream contain some items which do not test proficiency in judging. This has been recognized for several years. The fact that they do not test proficiency is no indication that the items should be eliminated. For example, data show that most participants judge bottle and cap about as well as the winning contestants. Deletion of this item might result in paying too little attention to the featuring of a clean and attractive bottle. A knowledge of the proper scoring of items of lesser importance is part of a student's training and should be retained. It is hoped that the time will come in the training of students when the item of flavor, for example, may be judged with less difference between the grades of the higher and lower ranking contestants than exists at present. When that time comes, the training in dairy products judging will be more effective.

In studying the data presented, the reader should keep in mind that the products used in the 1947 Collegiate Students' International Contest in the Judging of Dairy Products may have been of such a character that utilization of the entire normal range was not feasible, because the products may not have merited different scores. It should be emphasized that in any contest the "normal range" need not necessarily be used.

SUMMARY

1. The key to the success of the winning contestants lay in their abilities to evaluate the flavor of the dairy products more nearly in line with the judgment of the official than their competitors. Also, they approximated

more nearly the judges' decision in scoring and determining the body and texture of Cheddar cheese and vanilla ice cream. The three high contestants agreed that no body and texture defects were present in the butter samples scored.

2. The items of melting quality and color of ice cream, sediment, container and closure of milk, color and salt of butter, and color of cheese are not so important as flavor and/or body and texture in testing the judging proficiency of the participants in the contest.

3. With the exception of ice cream, contestants of the fourth quartile failed to score higher because of their inability to place a correct score on the flavor of the product.

4. Concerning the use of the suggested normal range in the scoring of each product, the following tendencies were observed: In the scoring of the flavor of butter, the contestants were more conservative than the judges. In the rating of the flavor of cheese and milk, the contestants and the judges were equally moderate. In the scoring of the flavor of ice cream, the contestants were much more conservative than the judge. All contestants were exceedingly cautious in placing scores on the body and texture of cheese and ice cream.

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MANOMETRIC MEASUREMENT OF THE GAS DESORBED FROM VACUUMIZED WHOLE MILK POWDER

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Packaging whole milk powder in an atmosphere extremely low in oxygen is recognized as being a safeguard in the prevention of off-flavors caused by oxidation of milk constituents. During spray drying of milk, gas is entrapped in the particles. This gas is not entirely removed by the ordinary single-stage gas-packing process. Therefore, packing milk powder in an atmosphere extremely low in oxygen becomes a problem of control of occluded oxygen, and possibly adsorbed oxygen, as well as one of proper technique in gas packing.

The origin of the entrapped gas has not been established definitely. Hetrick and Tracy (3) suggest that perhaps the oxygen dissolved in the milk is a factor in the amount of oxygen entrapped in the powder, but Coulter and Jenness (1) were unable to eliminate the entrapped gas in the powder particles by deaerating the condensed milk before drying.

The composition of the entrapped gas varies somewhat with the storage time of the dried milk in an atmosphere of air, according to Haller and Holm (2). Their results showed that the gas entrapped in the particles contained from 22 to 39 per cent oxygen.

Measurement of the amount of oxygen occluded has been done in a number of ways. Lea *et al.* (4) have derived a formula by which they calculate the milliliters of oxygen per gram of powder, knowing the initial per cent oxygen and the final per cent oxygen after equilibrium has been reached in the headspace gas of nitrogen-packed powder. Haller and Holm (2) used an apparatus with which they could remove the "sorbed" gases, measure their volume, and determine their composition.

If whole milk powder is put into a container and the container evacuated, the amount of gas that is removed will depend upon the temperature of the powder, the absolute pressure, the length of time of vacuumizing, as well as the physical characteristics of the powder itself. The occluded oxygen is not removed to any appreciable extent unless vacuumizing is continued for long periods of time. Use was made of this fact in this particular study to determine the amount of oxygen or gas entrapped in the powder.

The objective of this study was to measure the rate of desorption, the amount of gas held, and the composition of the gas "desorbed" from vacuumized whole milk powder by the manometric method. The results secured Received for publication May 14, 1948.

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by this method also were compared with those secured by the method of Lea *et al.* (4). Using the manometric method, the effects of aging the powder in the air before vacuumizing and of saturating the milk with carbon dioxide before drying on the amount of gas held during vacuumizing, and on the composition of the desorbed gas, was determined. It was hoped that by this procedure information on the origin of the entrapped gas could be secured.

EXPERIMENTAL METHODS

The whole milk powder used in this study was prepared in the laboratory by drying preconcentrated milk in the small pilot drier. The drier was a pressure-spray type with a capacity of approximately 30 lb. of powder per hour. The apparatus shown in figure 1 was used to measure the

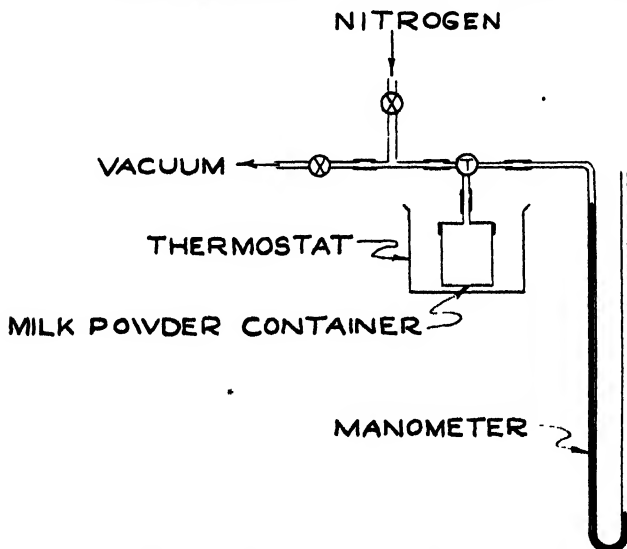


FIG. 1. Apparatus used to measure the gas desorbed from vacuumized whole milk powder.

rate of desorption and the amount of gas held. The composition of the gas desorbed from the vacuumized whole milk powder was determined by the method of Van Slyke and Sendroy (5). The whole milk powder container shown in the figure was calibrated up to the stopcock so that the packing density could be determined when the weight of milk powder was known. Capillary tubing was used for the connections and also for the manometer.

Whole milk powder was weighed into the cans so that the packing density in grams per milliliter of container volume was approximately 0.5. The manometer was affixed to the can and a three-way valve was set in such a way that the vacuum pump and the manometer both were connected

to the can of powder. By means of the vacuum pump, a vacuum was drawn on the system in approximately two minutes to an absolute pressure of 1-2 mm. mercury. The three-way cock then was turned in such a way that the can of powder was opened only to the manometer, and the vacuum pump was removed. As the entrapped air desorbed from the powder, the pressure increased. Absolute pressure measurements were made until the pressure became constant. All pressure measurements were made at 25° C. When the pressure measurements became constant, it was assumed that equilibrium conditions had been reached. Knowing the equilibrium pressure, the volume of the can, the weight of powder, and using a value of 1.31 for the density of air-free powder, the number of moles of gas, or volume of gas (*N.T.P.*) per gram of powder, which was held during the initial vacuumizing process, could be calculated. For this purpose, the following formula was developed.

$$\text{cc. gas/g.} = \left(\frac{22,400 P_e}{W R T} \right) \times \left(V_c - \frac{W}{1.31} \right)$$

Where P_e = the equilibrium pressure (mm. Hg)

W = " weight of powder in grams

V_c = " volume of the container (cc.)

T = " absolute temperature

R = " gas constant (62,400 cc. mm.)

1.31 = " assumed density of air-free milk powder in grams/cc.

When the equilibrium pressure was reached, the volume of gas was so small that a sample could not be removed for analysis. In order to determine the composition of the gas, nitrogen was admitted rapidly to a known pressure and then a sample of the mixed gas was removed for analysis. Blank determinations were made in which there was no milk powder in the can to determine the per cent oxygen in the nitrogen used. Knowing the per cent oxygen in the nitrogen used, the pressure of the nitrogen, the equilibrium pressure of the gas in the container, and the total pressure and the per cent oxygen of the gas in the container after the nitrogen was admitted, the percentage of oxygen in the gas that was desorbed from the sample of milk powder could be calculated by means of the following formula:

$$X_o = \frac{(X_T P_T) - (X_N P_N)}{P_o}$$

Where X_o = the per cent oxygen in the "desorbed" gas

X_T = the per cent oxygen in the mixed gas

P_T = the total pressure (mm. Hg)

X_N = the per cent oxygen in the nitrogen

P_N = the nitrogen pressure (mm. Hg)

P_o = the equilibrium pressure (mm. Hg)

It is recognized that this method is not an absolute one for measuring the amount of oxygen held by the particles during the vacuumizing process.

It does not include the adsorbed gas which may be present under equilibrium conditions. However, it is believed to show differences among different treatments of milk powder with respect to the amount of oxygen held during vacuumization.

RESULTS

Reproducibility of the method. In order to ascertain how closely values from a number of runs would agree, four determinations on the same powder were made. The results are shown in table 1. The absolute pressure measurements were reproduced closely. The same milk powder used

TABLE 1

Reproducibility of pressure measurements during desorption of gas from vacuumized whole milk powder

Time after vacuumizing (hr.)	Absolute pressure ($P_B - P$) at 25° C. (mm. Hg)			
	Trial 1	Trial 2	Trial 3	Trial 4
0	1	1	2	2
18	21	21	21	20
27	28	28	28	28
42	30	31	30	30
55	32	31	31	31
71	34	33	33	33
90	35	34	34	34
98	35	35	35	35
114	35	35	35	36
144	36	35	36	36
187	37	36	37	37 ^a
258	37	36	40	62
336	40	41	40	10
403	41	42	42	11
474	41	41	41	11

^a Leaker re-evacuated to 2 mm.

in this experiment also was packed in nitrogen gas and gas analyses were run on the headspace gas of the samples of powder until equilibrium was reached. The formula developed by Lea *et al.* (4) was used to calculate the milliliters of oxygen per gram of powder, as well as the milliliters of gas per gram of powder, and the values were compared to those secured by the manometric method. The results are listed in table 2.

From these results it can be seen that the two methods of determination check closely with respect to the milliliters of oxygen per gram, but not so well for the milliliters of gas per gram. The conclusion to be reached is that perhaps the difficulty lies in the composition of the gas desorbed with respect to per cent oxygen. Lea *et al.* (4) have assumed in their derivation that the gas entrapped in the particles contains 20.85 per cent oxygen. If the gas desorbed has a higher oxygen content, as data by the manometric method show, then one would expect to have a lower value for the milli-

liters of gas evolved per gram of powder. The assumption that the composition of the gas entrapped in the powder is 20.85 per cent oxygen is not significant when one uses this assumption to calculate the milliliters of oxygen per gram of powder. With the formula that they have derived, any error involved in this assumption would largely cancel out. However, in the calculation of the milliliters of gas held per gram, the value for the per cent oxygen in the gas desorbed becomes important.

With the manometric method, the values for the milliliters of gas per gram are more accurate than those representing the milliliters of oxygen per gram because the pressure can be measured accurately, but small variations in the per cent oxygen in the mixed gas make large errors in the

TABLE 2

A comparison of the manometric method and the method of Lea et al. (4) for determination of ml. of oxygen and ml. gas entrapped per gram of whole milk powder

Manometric method									
Trial	Vol.	W	P _s	P _N	P _T	%O ₂ (P _T)	%O ₂ gas desorbed	Ml. gas/g.	Ml. O ₂ /g.
1	477	238.5	43	712	755	2.21	33.8	0.064	0.0216
2	474	237.0	43	710	753	2.38	36.7	0.064	0.0235
Method of Lea et al.									
Time	% O ₂	Equilibrium % O ₂	D	d	ml. gas/g.	ml. O ₂ /g.			
(days)									
0	0.3								
4	2.01	2.19	1.31	0.50	0.113	0.0235			
7	2.24								
14	2.16								
26	2.33								

milliliters of oxygen. A difference of approximately 0.1 per cent oxygen in the gas mixture makes a difference of about 1.8 per cent in the oxygen content of the desorbed gas. However, the data are sufficiently accurate to show that the per cent oxygen in the desorbed gas is higher than that of ordinary air. This is in agreement with the results of Haller and Holm (2). One could speculate from this that the source of the entrapped air in the particles may be the gas that is dissolved in the milk before drying, or that oxygen is preferentially held in the cavities of the particle during vacuumizing.

Importance of gas entrapped in milk at time of drying. If the origin of the gas which is entrapped in the milk powder particles is the milk before drying, it was reasoned that the composition of the desorbed gas could be changed by changing the composition of the gas dissolved in milk. Two lots of powder were dried, one serving as a control and one in which

the carbon dioxide was bubbled into the condensed milk before drying. Both lots were spray dried as nearly alike as possible as far as pressures, temperatures, and nozzle sizes were concerned. Duplicate tests were run on both lots of powder when the powder was freshly prepared, and another set of duplicate runs was made after the powder was exposed to air for 24 hours after drying. The data for these runs are recorded in table 3

TABLE 3

Effect of saturating the condensed milk with carbon dioxide and age of powder before vacuumizing on the gas content and composition of the gases desorbed from vacuumized whole milk powder

Sample no.	Trial	Treatment	Vol. ^a	W	P _e	P _N	P _T	% O ₂ (P _T)
1	A	Control, packed immediately	477	238.5	29	721	750	1.75
	B	Leaker						
2	A	Control, packed 24 hr.	474	237	35	843	878	1.62
	B		475	237	35	853	888	1.54
3	A	CO ₂ added to milk, packed immediately	474	202	58	812	870	1.34
	B		479	210	57	800	857	1.20
4	A	CO ₂ added to milk, packed after 24 hr.	468	200	61	819	880	2.10
			469	200	62	808	870	2.06

Sample no.	% O ₂ desorbed gas	% CO ₂ (P _T)	% CO ₂ desorbed gas	Ml. gas/g.	Ml. O ₂ /g.	Ml. CO ₂ /g.
1	37.5	Leaker		0.043	0.0162	
2	33.4			0.052	0.0174	
	32.1			0.053	0.0170	
3	15.9	2.61	39.1	0.110	0.0175	0.0430
	13.8	2.75	41.3	0.104	0.0143	0.0429
4	26.3	2.19	31.6	0.116	0.0305	0.0367
	24.9	2.16	30.4	0.118	0.0294	0.0359

^a Vol. = Volume of container (cc.).

W = Weight of powder in grams.

P_e = The equilibrium pressure (mm. Hg).

P_N = Pressure of nitrogen (mm. Hg).

P_T = Total pressure (mm. Hg).

and the pressure changes by the manometric method are recorded in figure 2. The per cent oxygen in the air desorbed from the control powder again was higher than that in ordinary air. On aging the control powder before vacuumizing, the per cent oxygen in the gas desorbed decreased slightly, but the total volume of oxygen held per gram as well as the total gas per gram increased by this treatment. Similar results on the per cent oxygen in the air desorbed were secured by Haller and Holm (2). When powder is gas-packed after aging in air for 24 hours, the per cent oxygen in the headspace gas increases over that in powder gas-packed immediately after drying (1, 3). The results of this work seem to show that even

though the per cent oxygen in the desorbed gas decreases by aging the powder 24 hours before vacuumizing, the total amount of oxygen actually increases because the total amount of gas increases proportionately more than the per cent oxygen decreases.

The effect of milk and powder treatment on composition of desorbed gas and amounts of gas held by powder. When powder was vacuumized immediately after drying, the total volume of gas held per gram of powder was more than doubled by bubbling carbon dioxide into the condensed milk before drying. The composition of the desorbed gas also was altered. This would lend support to the belief that at least in part the air or gas

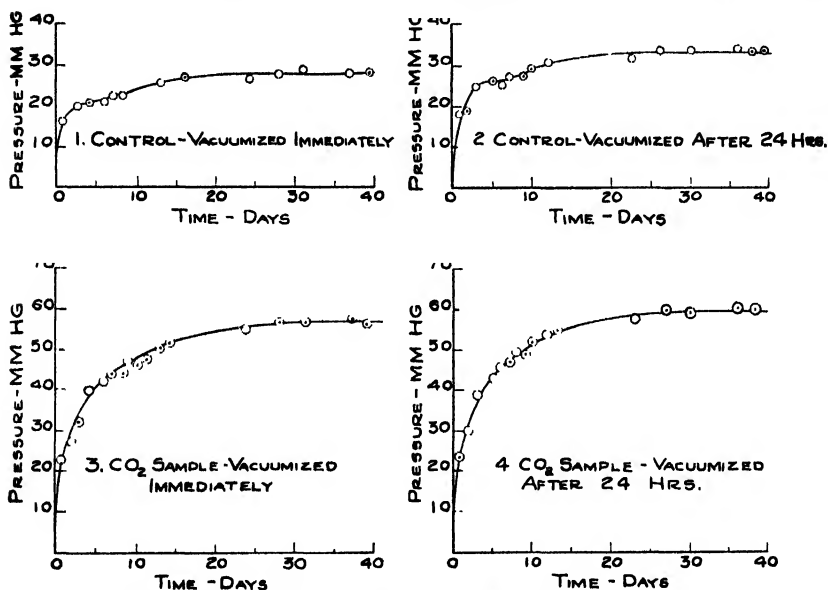


FIG. 2. Pressure changes during desorption of gas from vacuumized whole milk powder.

dissolved in milk is a source of the gas entrapped in the powder particles. Even though the total volume of gas held per gram by this treatment was much higher than in the control powder, the volume of oxygen held per gram was nearly the same as the control batch. When powder manufactured from condensed milk that had been saturated with carbon dioxide before drying was exposed to air for 24 hours before vacuumizing, a slight increase in total gas held per gram of powder was observed and almost a two-fold increase in the amount of oxygen held per gram was found. The carbon dioxide content of the powder was decreased by exposing this powder to air for 24 hours before vacuumizing. The increase which was observed in the total gas content by exposing milk powder 24 hours before

vacuumizing could be attributed to temperature and pressure phenomena, because the powder which was vacuumized immediately was warm and as the powder cooled, it is likely that more gas would be sorbed.

SUMMARY

A manometric method is presented for the determination of the amount of gas held and the composition of the gas desorbed from vacuumized whole milk powder. The amount of sorbed gases in dried milk varied with the methods of preparation and packaging. When freshly made powder was vacuumized after drying, the per cent oxygen in the gas desorbed from powder was higher than that of ordinary air. Upon exposure of powder to air for 24 hours before vacuumizing, the per cent oxygen in the gas desorbed was less, but not lower than the per cent oxygen in normal air. The total volume of oxygen held per gram of powder was increased by exposure of powder to air for 24 hours before vacuumizing. The composition of the gas desorbed and the total quantity of gas per gram of powder could be varied by saturating the milk with carbon dioxide before drying. This would lend support to the belief that, at least in part, the source of the gas entrapped in powder is the gas dissolved in milk.

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THE EFFECT OF AGITATION UPON THE LIVABILITY OF BOVINE SPERMATOZOA¹

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In the routine operation of most artificial breeding associations, diluted semen samples are transported from the central bull stud to the outlying inseminators, and often considerable distances are involved. Various means of transportation are employed and it is obvious that the semen samples are subjected to different amounts of agitation enroute.

Bretschneider (2) reported that the vigorous shaking of bull semen for 3 minutes destroyed motility. Spermatozoa from the normal ejaculate showed more resistance to shaking than did those secured from the testicle or epididymis. Smirnov-Ugrjumov (14) observed that the transportation of undiluted bull semen in thermos flasks at 15-20° C. for distances of 0.6 to 5.9 miles brought about a reduction in spermatozoan activity. Hronopulo (6) reported that transportation of undiluted bull semen did not affect the fertility of the semen during the first 4 hours of storage. After a storage period of 4 hours, however, the fertility of semen transported distances greater than 21.7 miles was markedly reduced. Ayyar (1) noted that hand shaking during transport killed bull spermatozoa.

Several workers have noted effects of agitation in connection with studies of semen physiology. Gunn (4) reported that periodic shaking of ram semen contained in rubber-stoppered test tubes was effective in providing the aeration which he considered necessary for maintenance of spermatozoan motility. Motility was maintained even when shaking was vigorous. On the other hand, Nagornyi and Smirnov (11) found that the resistance of ram spermatozoa to sodium chloride was decreased by continuous agitation. During the course of metabolism studies with ram and bull semen, Mann (10) observed that when a suspension of spermatozoa was shaken vigorously in the presence of air, the cytochrome enzyme within the cell was oxidized rapidly.

In the process of examining samples of diluted semen shipped to this laboratory from the several artificial breeding cooperatives in Pennsylvania, it was

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noted that the motility of the spermatozoa usually was lower in tubes of diluted semen shipped partially filled than in completely filled tubes. Poor livability often was evident, particularly in samples shipped in 14-ml. capacity test tubes which contained only 3-4 ml. of diluted semen. The decrease in semen quality observed may have been due in part to harmful effects of agitation in transit. Thus, the present study was proposed to determine the effect of mechanical agitation of diluted bull semen upon the livability of the spermatozoa.

EXPERIMENTAL PROCEDURE

Fifteen semen samples were obtained by means of an artificial vagina from three fertile bulls of the College dairy herd representing the Ayrshire, Brown Swiss and Holstein breeds. The 15 ejaculates were diluted at a constant rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. The diluter was composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g. of sodium citrate dihydrate in 100 ml. of water distilled over glass.

The effect of agitation upon spermatozoan livability was studied by subjecting diluted semen in three series of test tubes to mechanical agitation for 6, 12 and 24 hours. A fourth series received no mechanical agitation and served as the controls. Each of the series consisted of four test tubes (15 × 125 mm.), which contained 3.5 ml., 7.0 ml., 10.5 ml. and approximately 14.0 ml. of diluted semen. With these amounts of material the tubes were approximately one-quarter filled, half filled, three-quarters filled and filled to the bottom of the cork stopper. This experimental design made it possible to determine not only the effect of varying amounts of agitation but also the effect of agitation upon the livability of spermatozoa in test tubes containing different volumes of semen.

The test tubes were prepared by placing the desired volume of diluted semen in each sterile tube by means of a sterile pipette. The tubes were stoppered with sterile corks, and melted paraffin was applied to the juncture of cork and glass to complete the closure. The tubes of diluted semen were placed in a water bath at room temperature and gradually cooled to about 7° C. in a mechanical refrigerator. In order to maintain a temperature of from 5 to 10° C. during agitation and at the same time simulate field shipping conditions, each series of tubes to be agitated was packaged in refrigerated cardboard cartons. Methods and materials as described by Perry (12) were used in packaging the samples. Refrigeration was provided by 800 g. of ice contained in rubber balloons. A test tube containing water at 5° C. was packaged next to the tubes of diluted semen. When the cartons were opened, the temperature was determined by inserting a cooled thermometer (5° C.) into the tube of water. The control tubes of diluted semen were not packaged and were stored in a refrigerator at 5° C.

Agitation was provided by placing the shipping cartons on a mechanical agitator which operated at the rate of 76 oscillations per minute through a horizontal distance of 4 inches. The cartons were placed on the agitator so that the longitudinal axis of the test tubes was parallel to the horizontal axis of the

agitator frame. Following the prescribed period of agitation, the cartons were opened and the temperatures of the contents of those cartons subjected to 24 hours of agitation were determined. The samples then were placed in a 5° C. water bath and stored in a refrigerator maintained at that temperature.

In addition to motility estimations made before and after agitation, estimations were made every 2 days during the 20-day storage period. In order to minimize bias on the part of the observer making the motility estimations, randomized numbers were placed on the test tubes prior to agitation.

RESULTS

The 15 semen samples studied had a mean concentration of 1,141,000 spermatozoa per cubic millimeter, a mean initial motility of 63 per cent active spermatozoa and a mean methylene-blue reduction time of 12 minutes. The mean temperature to which the diluted samples were cooled prior to packaging was 7.1° C. and ranged from 5.5 to 8.9° C. The mean temperature of the samples after 24 hours agitation was 6.8° C., with a range of from 5.5 to 8.9° C.

The mean percentages of motile spermatozoa during 20 days of storage are presented in table 1. Each figure represents a mean of 15 ejaculates. Mechanical agitation of the partially filled tubes brought about a significant reduction in spermatozoan livability. The decrease in livability was related directly to the length of the agitation period. In addition, it was noted that the effect of agitation was related to the volume of semen contained in the tubes. Thus, the ability of the spermatozoa to remain motile during storage following agitation for 6, 12 and 24 hours was less in the one-quarter filled tubes than in the half filled tubes and less in the half filled than in the three-quarters filled tubes. When the tubes were completely filled, spermatozoan livability was not affected as

TABLE 1
Effect of mechanical agitation upon the livability of bull spermatozoa

Fullness of test tube	Length of agitation	Before storage	Per cent motile spermatozoa (15 ejaculates)				
			After storage at 5° C. for				
			4 days	8 days	12 days	16 days	20 days
Filled	(hr.)						
	0	63	49	43	32	17	5
	6	63	52	42	31	16	7
	12	63	50	41	29	16	7
Three-quarters filled	24	63	51	42	30	15	5
	0	63	51	39	28	17	7
	6	63	47	32	23	9	3
	12	63	43	31	16	6	3
Half filled	24	63	41	27	13	6	1
	0	63	51	42	26	13	7
	6	63	41	29	19	6	1
	12	63	38	27	13	4	0
One-quarter filled	24	63	36	19	11	4	1
	0	63	49	26	13	5	3
	6	63	39	21	9	3	0
	12	63	31	21	11	3	1
	24	63	27	10	5	2	1

markedly by agitation. Differences in livability of the spermatozoa also were obtained in the controls which received no mechanical agitation. Spermatozoa in the completely filled tubes maintained a significantly higher level of motility during storage than spermatozoa in one-quarter filled tubes.

Analysis of variance (table 2) involving 2,400 motility observations showed highly significant differences ($P = < 0.01$) between the two treatments, length of agitation (L) and fullness of tube (F), as well as between ejaculates and storage intervals. The interaction of the two treatments ($L \times F$) also was found to be highly significant, as were all other first and second order interactions.

TABLE 2
Analysis of variance of per cent motile spermatozoa during 20 days of storage following agitation for 0, 6, 12 and 24 hours

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	2,399	9,724	
Length of agitation (L)	3	304	101.33 ^a
Fullness of tube (F)	3	708	236.00 ^a
Storage intervals (S)	9	5,371	596.78 ^a
Ejaculates (E)	14	1,588	113.43 ^a
Interactions:			
$L \times F$	9	63	7.00 ^a
$S \times F$	27	100	3.70 ^a
$S \times L$	27	40	1.48 ^a
$L \times E$	42	42	1.00 ^a
$F \times E$	42	75	1.79 ^a
$S \times E$	126	437	3.47 ^a
$L \times F \times E$	126	138	1.10 ^a
$S \times L \times E$	378	171	0.45 ^a
$S \times L \times F$	81	62	0.77 ^a
$S \times F \times E$	378	225	0.60 ^a
Remainder	1,134	400	0.35

^a = Significant at the 1 per cent level.

According to the least mean differences required for significance, differences in livability between the partially filled tubes and the filled tubes were highly significant after 6, 12 and 24 hours of agitation. The differences between the filled tubes of semen which received no agitation and those agitated for 6 and 12 hours were not statistically significant. However, after agitation for 24 hours, the differences barely reached significance at the 5 per cent level. Highly significant differences were found between the one-quarter filled and the completely filled tubes which were not subjected to mechanical agitation, while the differences between the filled tubes and the half and three-quarters filled tubes were not statistically significant.

DISCUSSION

The present study was designed to determine the effect of agitation upon the livability and metabolism of bovine spermatozoa. It was hoped that the latter information would be useful in explaining results obtained in the livability phase. Because of difficulties in obtaining reliable results with the methods employed

in the metabolism study, this phase of the problem was not completed. However, it is possible that the detrimental effect of mechanical agitation upon spermatozoan livability is related to the amount of atmospheric oxygen in the test tubes.

The results of the livability study showed that irrespective of the length of agitation an inverse relationship existed between the amount of air in the test tubes and the livability of the spermatozoa. However, decreases in livability were greatest in those tubes which contained the largest volumes of air and which were agitated for the longest periods of time. When a minimum of air was present (filled tubes) agitation did not affect markedly the livability of the spermatozoa. These observations of the detrimental effects of aeration are supported by the statistical analysis of these data. As shown in table 2, all of the sources of variation were found to be highly significant. However, the table shows that a greater mean square was obtained for fullness of tube than for length of agitation. The mean square for the interaction, length of agitation \times fullness of tube, also was larger than the mean square for any of the other first order interactions.

It has been shown (3, 8, 13, 16 and others) that although a certain volume of oxygen normally is utilized in the metabolism of bovine spermatozoa, respiration is not essential for motility (7). While the exact role of oxygen in the metabolic processes is not clear, there is evidence that an excess of oxygen, in certain instances, may be detrimental to spermatozoan livability. Walton (15) concluded that protection of semen against exposure to air may be beneficial to livability and recommended that the semen be covered with a layer of medicinal paraffin oil. Willett and Salisbury (17) also found that motility was maintained longer during storage when semen was covered with a layer of mineral oil. MacLeod (9) found that oxygen was detrimental to the motility of human spermatozoa. Recently Salisbury (13) reported that bovine spermatozoa in low concentration were harmed by oxygen. Based on these findings, it is possible in the present study that excess aeration of the diluted semen was responsible, in part, for the decreases in livability obtained.

CONCLUSIONS

1. In the routine shipment of partially filled tubes of diluted bull semen a decrease in spermatozoan livability may be encountered due, in part, to the effect of agitation.

2. The harmful effect of agitation may be minimized by completely filling the test tubes with diluted semen.

3. On the basis of the data obtained in this experiment, it seems advisable to ship diluted semen in completely filled test tubes of different capacities to meet the individual requirements of the inseminators.

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HEAT INACTIVATION OF MILK PHOSPHATASE IN DAIRY PRODUCTS

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A description of the phosphatase test and the modifications that make it applicable to various dairy products to determine the adequacy of pasteurization was published in 1947 (12). In a later publication (13), it was pointed out that negative results with the phosphatase test indicate that the pathogenic organisms that may have been present were destroyed.

With a phosphatase test available that is quantitative over a wide range (from no heating to complete inactivation) and that is relatively sensitive and precise, and with considerable data available in the literature on the thermal death points of various microorganisms, it seemed desirable to determine experimentally the heating conditions necessary to produce various degrees of inactivation, including complete inactivation, of the phosphatase enzyme in milk and in some other dairy products. Such results would be useful in formulating pasteurization standards for various dairy products besides milk.

This report describes a laboratory pasteurizer that was used in these studies for controlling the temperature and duration of heating with a high degree of precision, and presents the results of phosphatase-inactivation experiments on whole milk, skim milk, cream (20 and 40 per cent fat content), ice cream and sherbet mixes, Cheddar cheese and cheese mixtures with emulsifiers or various other substances added.

Precise control of the temperature and duration of heating is necessary for reliable results in studying experimentally the thermal destruction of bacteria and phosphatase. North and Park (10), determining thermal death points of tubercle bacilli, used a laboratory pasteurizer fitted with a tubular metal coil immersed in a bath at a controlled temperature. The milk, heated to the desired temperature, was inoculated and was allowed to flow by gravity into the tubular coil where it was held at the experimental temperature. This method offered relatively more precise results than older methods, in which samples, at room temperature, were placed in glass tubes, inoculated and the tubes then placed in the heated bath. It reduced the heat lag, allowed more precise control of the heating time, and eliminated surface cooling.

Some investigators, heating samples in glass tubes (11) or in metal containers (6, 8), used a series of three water baths—the first one at a temperature below the desired holding temperature in order to preheat the samples uniformly, the second one at a temperature somewhat higher than the holding temperature in order to decrease the time lag by heating the samples rapidly, and the third one at the desired holding temperature. Despite the need for increasing the temperature rapidly, there appears to be considerable possibility of over-heating some particles

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of the sample when any part of the equipment containing the sample is heated at a temperature higher than the specified experimental temperature.

Gilcreas and O'Brien (4) recently have described a laboratory pasteurizer, used in bacteriological experiments, fitted with an automatic inoculating and sampling device actuated by an electric timer. With this equipment, inocula can be injected into a medium that is held constantly at the desired temperature, and samples likewise can be withdrawn with time intervals controlled very accurately. Although the heating-time lag is thus greatly reduced, this equipment could not be used for obtaining phosphatase-test data, because in phosphatase experiments it is necessary to control the heating conditions of the entire sample rather than only the portion used as an inoculum in bacteriological experiments.

EXPERIMENTAL METHODS

Attempts were made at first to make use of a Mallory-type heater, with the heating tube surrounded by steam, but it was difficult to determine and control the temperatures of small samples with the desired precision without installing a highly sensitive thermocouple and without other modifications of the control of the temperature. The results of the phosphatase tests on the samples heated in this manner were not sufficiently consistent for this purpose.

A laboratory pasteurizer, illustrated in figure 1, was assembled and used for heating fluid samples. The pasteurizer comprised a thin-wall tubular metal coil 30 feet long and one-eighth inch internal diameter, with a metal holding chamber connected at the lower end. This assembly was immersed in a water bath fitted with heaters, stirrer and thermoregulator, which controlled the temperature of the bath with a variation not greater than $\pm 0.2^\circ$ F. The pasteurizer coil as used at first was fitted at the midway point with a T-tube connection (not shown in diagram) in which a four-junction thermocouple in thin-wall glass tubing was installed. A similar thermocouple was installed permanently through the stopper in the holding chamber. Temperatures were determined by means of a Leeds and Northrup type K potentiometer. Six mercury thermoregulators set at approximately 5° intervals between 142 and 168° F. were used. A special thermometer, reading 140 – 180° F. in 0.1° intervals, calibrated against one that had been checked at the National Bureau of Standards, was used in the bath and for calibrating the thermocouples. Heating-time periods were regulated with an electric stopclock calibrated in seconds.

To reduce the heating-time lag uniformly, the samples first were warmed in the phosphatase-test bath to 99 – 100° F., and then forced into the pasteurizer coil at high speed by means of air pressure. A suitable initial pressure, controlled by pressure regulator *A* (fig. 1) set at between 9 and 12 inches of mercury for milk and 12 to 15 inches for cream, was built up by means of a slow flow of air into flask *B*. Twenty to thirty-five ml. of warmed sample was put into sample chamber *S*₁, which was stoppered, and stopcock *SC* was turned to build up the pressure on the sample. Then rubber inlet tube *S*₂ was opened and the stopclock was started. The pressure forced the sample into and through pasteurizer coil *C* and into the bottom of holding chamber *HC*. The lower clamp on the inlet tube immediately

was placed below the water line in the bath and the inlet tube was closed. Under these conditions, the largest decrease of temperature that could be detected by means of the thermocouple at the midway point in the pasteurizer coil during flow was 0.2°F. , and such decrease was only momentary. No change of temperature in the holding chamber could be detected.

At the ends of specified time periods, 2- to 3-ml. test samples were withdrawn quickly from the holding chamber through narrow-bore glass tubes *D*, by means of suction, into test tubes immersed in ice water. To allow for lag, a correction of 3 seconds for the smaller samples and 4 seconds for the larger ones was subtracted from the total heating time from the beginning of flow. The heating times thus corrected and recorded in the graphs are believed to be the averages of the periods during which the entire sample of each product was held at the experimental temperature. This seems a more feasible time to record than the over-all heating time.

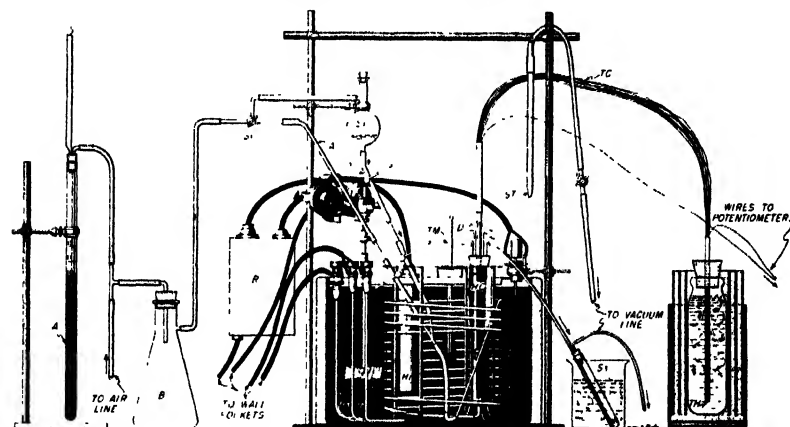


FIG. 1. Laboratory pasteurizer. *A*—adjustable air-pressure regulator. *B*—air-pressure reservoir flask, 1 liter. *SC*—three-way stopcock. *S*₁—sample chamber. *S*₂—sample inlet tube. *A*₁—air inlet tube for agitating sample. *C*—pasteurizer coil, 30 ft. long, $\frac{1}{4}$ in. internal diameter (tin alloy). *HC*—metal holding chamber, insulated above water line. *D*—glass tubes for removing samples. *S*₃—samples collected in tubes in ice water. *WB*—water bath. *H*—heaters, 300-watt: *H*₁ operated by thermoregulator, *H*₂ on constantly, *H*₃ for increasing bath temperature rapidly. *E*—stirrer. *TM*—thermometer. *TR*—thermoregulator. *R*—relay. *TC*—thermocouple, copper-constantan, 4-junction in series. *TH*—thermocouple reference junction in insulated ice bath. *ST*—suction tube for cleaning.

For the fluid samples that were heated at the higher temperatures and for the shorter periods, the time required to heat the entire sample was decreased by using only 20 ml. of sample and increasing the initial pressure to as high as 12 inches of mercury for milk and 15 inches for cream. Under these conditions it required not more than 4 seconds for the entire sample to pass into the pasteurizer coil and 6 seconds from the beginning of flow for the front end of the sample to reach the holding chamber.

To avoid the difficulty caused by the cream rising or a protective pellicle form-

ing on the samples, mentioned by Smith (14) and by North and Park (10), the samples were agitated by means of air. As soon as the sample had passed in and the clamp on the sample inlet tube S_2 had been closed, stopcock SC was turned and the clamp on air inlet tube A_1 was released, permitting air to flow slowly through the pasteurizer coil and to agitate the sample with two or three bubbles per second. The temperature of the air above the sample was found to be the same as that of the sample. The temperatures in the holding chamber and in the sample did not fluctuate as much as those in the water bath.

At least six test samples, each heated for a different period of time, were obtained of each product heated at each temperature. Sufficient test samples were obtained so that at least one was negative (zero value) and at least four were positive. If the results did not meet these conditions, the experimental heating was repeated at the same temperature but under a modified set of time conditions. For example (fig. 2), a 20-ml. sample of whole milk was heated at 158.2° F. and test samples were obtained first that were heated for 42, 50, 60, 75, 90, and 110 seconds, respectively; the first two yielded phosphatase values less than 5 units per ml. and the last four were negative. The heating was repeated and test samples obtained that had been treated for 24, 27, 30, 35, and 42 seconds; the first yielded more than 40 units and the last yielded less than 2 units per ml.

The milks tested were fresh and were taken from the composite milks obtained from a large herd. The tests on creams and skim milks were run on samples prepared by separating portions of the same whole milks that were tested. The ice cream mixes contained 15 per cent fat, 8.5 per cent milk serum solids, 14 per cent sugar, and 0.3 per cent stabilizer, and had an average pH of 6.22. The sherbet mix contained 4 per cent fat, 3.5 per cent serum solids, 25 per cent sugar, and 0.3 per cent stabilizer, and had a pH of 6.30 before pasteurization and the addition of acid. The fat and phosphatase present in the mixes were from the raw cream, additional serum solids being furnished by condensed skim milk. The Cheddar cheese, which was made from raw milk, was of normal composition and between 1 and 2 months old when tested. It had a pH value of 5.29 and a phosphatase value of 3,450 units per g. The original phosphatase values of the other products were normal and within the ranges stated earlier (13).

In addition to tests on fluid products, 3-g. samples of ground cheese were placed in heat-sealing metal foil envelopes, and these were pressed to a uniform thickness of 1 mm., warmed in the phosphatase-test bath and immersed in the pasteurizing bath. With a thermocouple coated with shellac and Miracle adhesive and sealed in with such cheese samples, the time required for the temperatures of the samples to reach within 0.25° F. of that of the bath at 150° F. was found to be approximately 18 seconds. A correction of 18 seconds was subtracted from the total heating time.

To determine the effects of pH on the inactivation of the enzyme by heat, 5-ml. portions of a sample of milk (a mixture of 10 per cent of raw milk with 90 per cent of pasteurized milk) were placed in a series of 12 test tubes, various quantities of normal acid or alkali were added—i.e., from 0.35 ml. of acid to 0.1 ml. of alkali, yielding a step-wise pH range of 4.09 to 8.42—the pH values were

determined, the samples were funneled into clean tubes so that there was no liquid on the inner surfaces of the tubes above the samples, and they were placed in a rack, warmed to 99–100° F., and then heated with agitation in a water bath at 140° F. for 5 minutes. Two and one-fourth minutes additional time was allowed for the temperatures of the samples to reach 139° F. The samples were cooled in

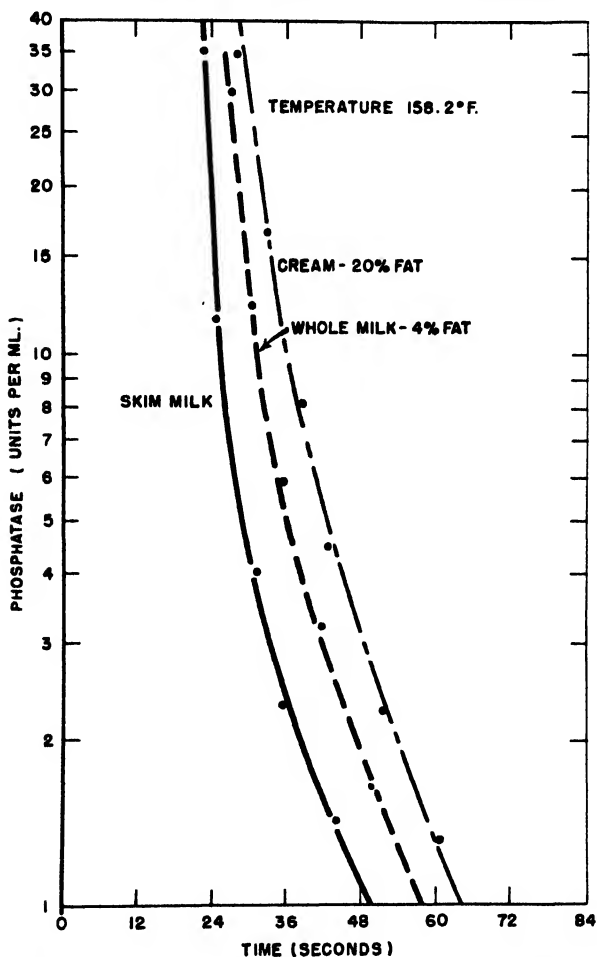


FIG. 2. Effects of duration of heating, at one temperature, on inactivation of phosphatase in skim milk, whole milk, and cream.

ice water, titrated back to the original pH with alkali or acid and tested for phosphatase activity. Corrections were made for the volumes of added acid and alkali.

Phosphatase tests were made by the method described earlier (12), and determinations of the intensity of the color were made with a Klett-Summerson photoelectric colorimeter with round matched tubes. Samples yielding less than 1 unit

per 1 ml. or per 0.5 g. of product in the test were considered negative, since such small values are difficult to determine accurately, even with a colorimeter, because of slight possible variations in the readings made on the controls.

RESULTS

The time-temperature inactivation results are summarized graphically. Figure 2 shows test data obtained on skim milk, whole milk, and 20 per cent cream, all were heated at 158.2° F., and illustrates the method of plotting the results. By conducting a series of tests on samples heated at a sufficient number of dif-

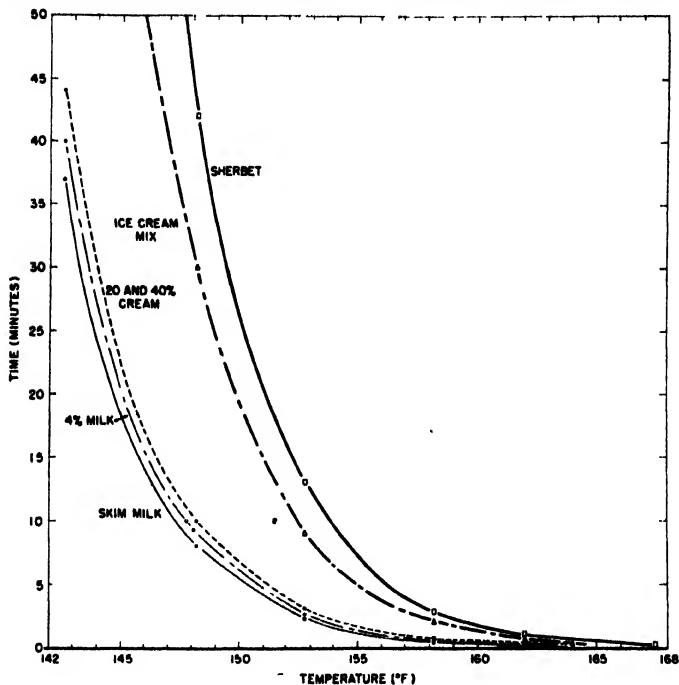


FIG. 3. Times and temperatures of heating required to reduce phosphatase activity to 2 units per 0.5 ml. (4 units per ml.) in different dairy products—test data plotted on an arithmetic scale.

ferent time periods, and thus plotting the data, it was possible to determine the heating times required at this temperature to produce zero values and also different degrees of inactivation.

With the phosphatase values plotted on a logarithmic scale and the duration of heating on an arithmetic scale (fig. 2), the curves in all experiments deviated from a straight-line course in a direction that indicates a marked decrease in the rate of inactivation as the time of heating is prolonged—i.e., the rate of destruction of the enzyme by heat is most rapid at first and diminishes greatly with time. The curves prepared in this manner from data obtained at the lower temperatures

intersect the horizontal axis at a narrow angle, and the curves from data at the higher temperatures intersect the horizontal axis at a wide angle, showing that complete destruction is approached more slowly at low temperatures than at high temperatures.

By means of a similar series of experiments at each different temperature, data were obtained and plotted to determine the heating conditions just sufficient to reduce phosphatase activity to a value of four units per ml. in skim milk, whole milk, 20 and 40 per cent cream, ice cream mix, and sherbet mix. With data plotted arithmetically, as shown in figure 3, it is difficult to evaluate accurately the

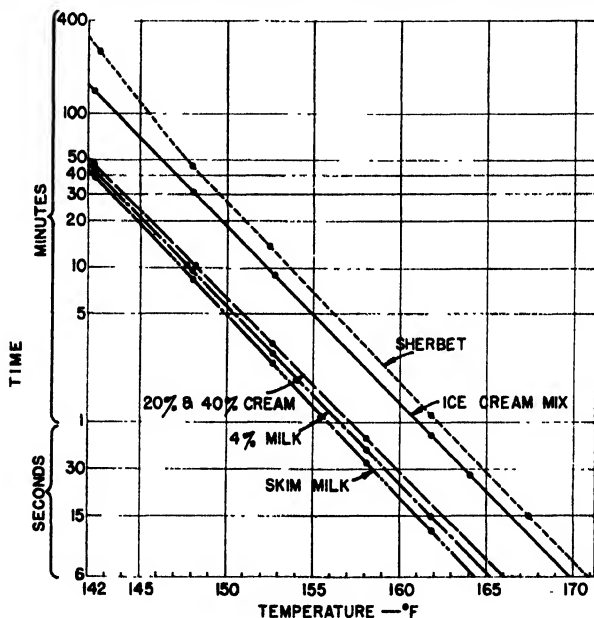


FIG. 4. Times and temperatures of heating required to reduce phosphatase activity to 2 units per 0.5 ml. (4 units per ml.) in different dairy products—data the same as in figure 3, but plotted on a semi-logarithmic scale.

heating times required in the high-temperature range. With the same data plotted on semi-logarithmic graph paper, however, as shown in figure 4, the points for each product form a straight line and the plot is readily usable.

The straight-line course of the time-temperature inactivation data conforms with results obtained earlier by Holland and Dahlberg (8) and Marquardt and Dahlberg (9) in studies on the effect of heat on cream layer volumes; with North and Park's results, as interpreted by Dahlberg (3), on the killing of tubercle bacilli; and with Hening and Dahlberg's (6) and Holland and Dahlberg's (8) results on the killing of *Escherichia coli*, the inactivation of phosphatase, and the effects on other properties. Mathematical equations pertaining to the straight-line pattern of time-temperature effects on a semi-logarithmic scale, produced in heat-

whole milk, about 0.7°F. higher for 20 and 40 per cent cream than for whole milk, about 4.5°F. higher for ice cream mix than for whole milk and about 5.7°F. higher for sherbet than for whole milk. The time required, at 143°F. , was about three times as long for ice cream mix as for whole milk.

Results obtained in tests on Cheddar cheese and on mixtures of cheese with emulsifying salts, alkalies, lactose or water, are shown in figure 5. Phosphatase was inactivated at considerably lower temperatures and shorter holding times in Cheddar cheese than in milk, *e.g.*, to a value of 12 units per g. at 130°F. for 13 minutes and at 140°F. for slightly less than three-fourths minute in cheese having a pH of 5.29. Mixing sodium carbonate or sodium hydroxide with the cheese to increase the pH had some effect in stabilizing the enzyme against heat inactiva-

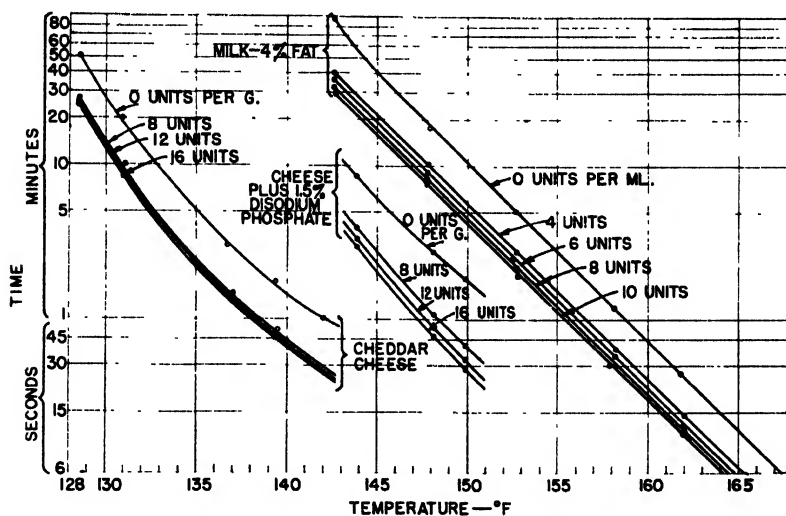


FIG. 6. Times and temperatures of heating required to cause various degrees of inactivation of phosphatase in milk, in Cheddar cheese, and in Cheddar cheese with 1.5% of *anhydrous* disodium phosphate added.

tion. Adding emulsifiers, such as sodium citrate or disodium phosphate, in quantities that affected the pH less, had a greater effect in stabilizing the enzyme. For example, in cheese with 1.5 per cent anhydrous disodium phosphate added (pH 5.56), a temperature of 150°F. for approximately 0.5 minute was needed to reduce the activity to 12 units per g. The addition of lactose increased the stability of the enzyme. The addition of water decreased its stability slightly.

Data showing the temperatures and times found necessary to produce various degrees of inactivation in milk and in cheese are shown in figure 6. As pointed out above, the last few remaining units were found to be the most difficult to inactivate. Differences between zero and four units were found to indicate considerably more heating than differences between four and eight units per ml. A pasteurization criterion of zero for milk, with this test, apparently would be too

severe, because it would require heating at temperatures several degrees higher than the temperatures specified in present pasteurization standards.

Figure 7 shows the effects of the pH values, at the time of heating, on the inactivation of the enzyme in milk. The heat stability was greatest when the pH was within a range of 6.5 to 7.4. In samples in which the pH was decreased progressively below 6.5, heating at 140° F. for 5 minutes decreased the activity markedly, until at pH 5.1 this heating reduced the activity to zero. There was a similar but less marked decrease in heat stability of the enzyme with increases in alkalinity beyond approximately pH 7.4.

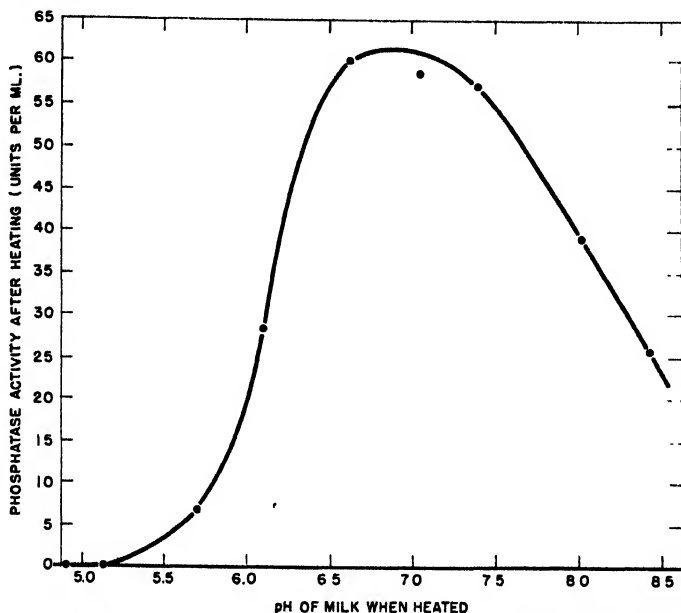


FIG. 7. Effects of pH on heat inactivation of milk phosphatase—mixture of 10% raw and 90% pasteurized milk; original phosphatase activity of mixture 195 units per ml.; heated for 5 minutes at 140° F.

DISCUSSION

Smith (14) showed that at 140° F. a considerably longer time is required to destroy tubercle bacilli in the pellicle that forms on the surface of milk than in the milk itself. Brown and Peiser (1) demonstrated that the temperature required, for a given period of time, to kill certain strains of *Streptococcus lactis* and of *E. coli* in cream is higher than that required to kill them in milk. Hening and Dahlberg (6), experimenting on the destruction of *E. coli*, inactivation of phosphatase, effect on flavor and effects on certain other properties, concluded that the present standards for pasteurization of milk are not adequate for cream. In addition to the protective effect present in cream, others (2) have shown that sugar also increases the stability of the enzyme against heat and present results

(fig. 4) corroborate this conclusion. Caulfield and Martin (2) reported that a temperature of 150° F. for 15 to 25 minutes was required to produce negative tests in ice cream mixes, and Hahn and Tracy (5) obtained similar results. It is evident that neither the present pasteurization standards for milk nor higher standards proposed by Hening and Dahlberg (6) for cream are adequate for ice cream and sherbet.

The heat stability of the enzyme is not as great in cheese as in milk. Although the stability is increased when the concentration of fat is increased (see data for cream, fig. 4), it will be noted that it is decreased much more by acidity when the pII is reduced (fig. 7) to values found normally in cheese. The acidity apparently has a predominant effect on the heat stability.

A large number of samples of process cheese, cheese foods and cheese spreads have been tested for phosphatase in these laboratories, and all of them have yielded zero values. The heating conditions used commercially in processing these products should be, and evidently are, adequate to accomplish the purposes of pasteurization.

In manufacturing cottage cheese curd from raw skim milk, a large proportion of the enzymic activity—frequently more than 80 per cent and sometimes nearly 100 per cent—is lost during manufacture. This decrease is attributed to the fact that the curd is heated, usually for a considerable period of time, after acidity has developed.

Experimental results have shown that the heat-inactivation reaction which phosphatase undergoes at the pH of normal milk is irreversible. On the other hand, when partial inactivation occurs only because of the development of acidity, the activity can be largely restored by adding sufficient alkali—*e.g.*, mixing 1 ml. of a 1.25 per cent aqueous solution of anhydrous sodium bicarbonate with 0.5 g. of cottage cheese—to increase the pII to approximately 7, and allowing the mixture to stand for several hours before testing. In the case of cottage cheese curd that has been washed thoroughly during manufacture, the presence of traces of added magnesium stimulates this reactivation, and the presence of magnesium and zinc stimulates it more.

SUMMARY

A laboratory pasteurizer is described, for controlling the heating temperatures and time accurately in pasteurization experiments.

Phosphatase test data for samples heated at any specific temperature for various periods of time show that the rate of destruction of the enzyme by heat is very rapid at first and diminishes to a relatively very slow rate with time. The experimental data for phosphatase destruction show that, in tests on milk and other fluid dairy products, a straight line results when the logarithms of the times of heating are plotted against the corresponding temperatures.

Holding periods required in this test to reduce phosphatase activity to four units per ml. of whole milk were: 37.5 minutes at 143° F., 30 minutes at 143.7° F., 24 seconds at 160° F., and 15 seconds at 161.8° F., respectively. The temperature required to produce a negative phosphatase test in any given time generally was

found to be about 0.7° F. lower for skim milk than for whole milk, about 0.7° F. higher for 20 and 40 per cent cream than for whole milk, about 4.5° F. higher for ice cream mix than for whole milk, and about 5.7° F. higher for sherbet than for whole milk. The time required, at 143° F., was about three times as long for ice cream mix as for whole milk.

Phosphatase was inactivated at considerably lower temperatures and shorter holding times in Cheddar cheese than in milk—*e.g.*, at 130° F. in 13 minutes and at 140° F. in slightly less than three-fourths minute in cheese at pH 5.29. Mixing alkalies with the cheese to increase the pH had some effect in stabilizing the enzyme against heat. Adding emulsifiers had a greater effect, as the temperature required to produce a negative test in approximately 0.5 minute was 150° F. when 1.5 per cent anhydrous disodium phosphate was added and the pH of the mixture was 5.56. The addition of lactose to cheese increased the stability of the enzyme; the addition of water decreased its stability slightly. Experiments on milks adjusted to different pH levels and heated showed that the milk phosphatase was most stable towards heat when the reaction was within a range of pH 6.5–7.4. Heating at lower or higher pH levels produced more rapid inactivation.

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THE ADAPTABILITY OF TWO STRAINS OF LACTIC STREPTOCOCCI TO GROWTH IN THE PRESENCE OF HOMOLOGOUS BACTERIOPHAGE¹

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Several investigators have reported on the adaptation of cultures of lactic streptococci to growth in the presence of bacteriophage. In all cases, the resistant cultures developed proved to be susceptible to other races of bacteriophage. In the present study, an attempt was made to determine more specifically how long it takes to adapt cultures of lactic streptococci to homologous bacteriophage, and after a strain of organisms has been made resistant, how long this characteristic will persist. Data from single trials, using two cultures of lactic streptococci and their homologous races of bacteriophage, are presented in this paper.

REVIEW OF LITERATURE

Whitehead and Hunter (5) found that a resistant culture of lactic streptococci developed by the action of bacteriophage on a sensitive strain was susceptible to attack by a new race of bacteriophage. They suggested that this type of action lends support to the theory that the bacteriophage is a product of the organism. Nelson and Hammer (3) isolated bacteriophage-resistant strains of *Streptococcus lactis* from the secondary growth of a culture upon which an inhibitory principle obtained from "slow" butter cultures had acted. Later work by Nelson *et al.* (4) showed that the secondary-growth organisms, which were not sensitive to the strain of inhibitory principle used, still were sensitive to other races of bacteriophage. Experiments by Anderson and Meanwell (1) indicate that bacteriophage-resistant strains of lactic streptococci can be developed, but on reintroduction into factory use these cultures become susceptible to secondary races of bacteriophage. Hunter and Whitehead (2) state that resistant strains of lactic streptococci usually develop sometime between 24 and 48 hours after bacteriophage has caused the lysis of a sensitive strain of organisms.

MATERIALS AND METHODS

Origin of cultures. The culture designated as H. P. is a strain of *Streptococcus cremoris* secured from the Dairy Research Institute in New Zealand. Culture no. 4 is a strain of *S. lactis* from the culture collection at Iowa State

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College. Homologous strains of bacteriophage for each were received with the cultures.

Preparation of cultures. The cultures were propagated in autoclaved re-constituted commercial dry milk solids not fat used at the rate of 90 g. per 1,000 ml. of distilled water. Transfers were made either daily or on alternate days. The cultures were incubated at 25° C. until coagulation occurred, after which they were refrigerated at 0° C. Cultures for use on any particular day were prepared by using a 1 per cent inoculum into milk late in the afternoon of the previous day.

Preparation of the bacteriophage filtrate. Sterile milk was inoculated with a milk culture of the test organism using a 1 per cent inoculum. At the same time, a few drops of whey filtrate containing the homologous bacteriophage were added. After incubation at 25° C. until coagulation occurred, usually 48 to 72 hours, the whey was filtered through a sterile Seitz filter. The resulting bacteria-free filtrate was transferred aseptically to a sterile container and stored at 0° C. until used. Several filtrates of the bacteriophage under study were prepared during the course of the experiment in order to have on hand a filtrate of maximum titer for use with each series.

Preparation of serial dilutions of bacteriophage. Serial dilutions of the whey filtrate containing bacteriophage were made directly into sterile milk. Dilutions ranging from 10^{-2} to 10^{-10} were used. The final volume, in all cases, was 100 ml. contained in a screw-capped 6-ounce prescription flask.

Titrate acidity determination. Titratable acidities were determined on weighed 9 g. samples, using 0.1 N NaOH and phenolphthalein as the indicator. The results were expressed as per cent of lactic acid.

EXPERIMENTAL

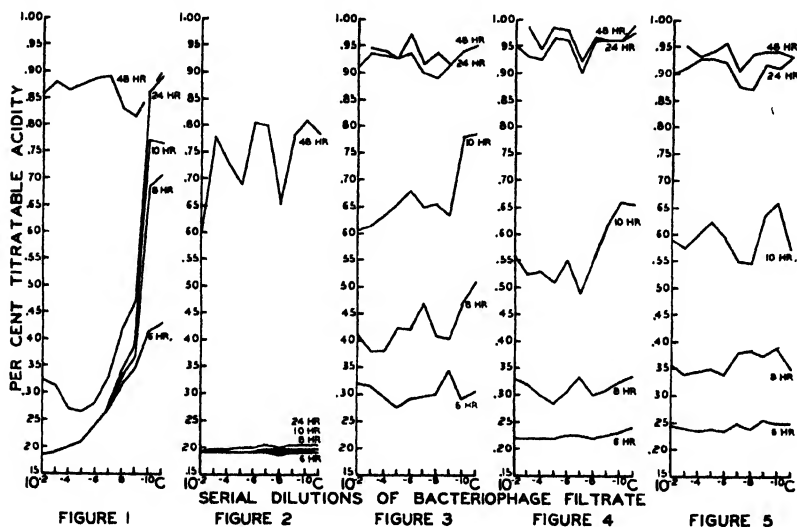
Adaptability of strain H. P. to the homologous bacteriophage. This experiment included five successive culture series. The first series consisted of ten flasks each containing 100 g. of autoclaved skim milk inoculated with a 1 g. amount of coagulated milk culture. These aliquots were dispensed aseptically from a stock flask in which the milk and the inoculum had been mixed thoroughly by vigorous agitation for a period of 1 minute. To the first nine of these flasks were added serial dilutions of the homologous bacteriophage whey filtrate ranging from 10^{-2} to 10^{-10} , inclusive. The tenth flask received no bacteriophage and served as a control to show acid production of the bacteriophage-free culture. The whole series was warmed to 30° C. in a water bath, after which the flasks were placed in an incubator adjusted to that temperature.

In the general plan of the experiment, the culture containing the lowest dilution of bacteriophage filtrate permitting coagulation of the milk after 24 hours of incubation was used to inoculate the flasks of the succeeding series. However, with series 2 it was necessary to wait 48 hours to secure a coagulated inoculum for use in the next series, as all cultures of this series, including the control, still were inhibited markedly after 24 hours by the residual bacteriophage carried over with the inoculum from series 1. Serial dilutions of the

homologous bacteriophage filtrate also were added to the flasks in all of the series by the method used in preparing series 1.

A 9 g. portion for acidity titration was withdrawn with a sterile pipette from each of the flasks after 2, 4, 6, 8, 10, 24 and 48 hours of incubation. The portions from the flasks in each series always were weighed and titrated in the same order, so as to maintain as closely as possible the desired time interval between each set of titrations.

Two series a week were started in this experiment, on Tuesdays and Saturdays, thus making one 3-day and one 4-day interval between the series of each week. The culture selected from each series for use in the succeeding series



FIGS. 1 through 5. Adaptability of strain II. P. to the homologous bacteriophage. Amount of acidity developed at 30° C.

was stored in the refrigerator at 0° C. until it was needed to make the necessary inoculation.

The data from the titrations after 6, 8, 10, 24 and 48 hours of incubation of the milk cultures are shown in figures 1 through 5. The data from the 2- and 4-hour titrations were not graphed because they did not show any significant change or differences between the various cultures containing serial dilutions of bacteriophage.

The data from series 1 (fig. 1) show that the titer of the bacteriophage filtrate used was at least as high as 10^{-9} and that the higher the serial dilution of bacteriophage filtrate added, the less was the degree of inhibition of the organisms in the culture.

The culture used to inoculate the flasks of milk of series 2 was from the 10^{-10} serial dilution of series 1. It is apparent from the data of series 2 (fig. 2) that considerable residual bacteriophage had been carried over in the inoculum from

the culture of series 1. The fact that the control culture, which had received no additional bacteriophage filtrate, was inhibited to the same extent as the others in the experimental series would seem to justify this conclusion.

The data in figure 2 also indicate that bacteriophage-resistant strains of this culture were not developed when the organisms were growing actively in the presence of a dilute concentration of bacteriophage. If any resistance to the bacteriophage had been built up in the culture used to inoculate the flasks of milk for series 2, these organisms would not have been inhibited so markedly after they had been transferred to new milk. The recovery of the organisms from the inhibiting effects of a high concentration of bacteriophage, sometime between the 24- and 48-hour titration intervals, indicates that bacteriophage-resistant strains are developed when the organisms are prevented from growing by the presence of a sufficient concentration of a homologous bacteriophage. No satisfactory explanation has been found for the rather wide and inconsistent variations in the titratable acidities after 48 hours of incubation of the cultures in series 2.

The data from series 3 (fig. 3) show that strains with some degree of bacteriophage resistance were developed sometime near the end of series 2, because the rate of acid production of nearly all cultures containing added bacteriophage was much more rapid at the 6-, 8- and 10-hour titration intervals in series 3 than was the case in either series 1 or 2.

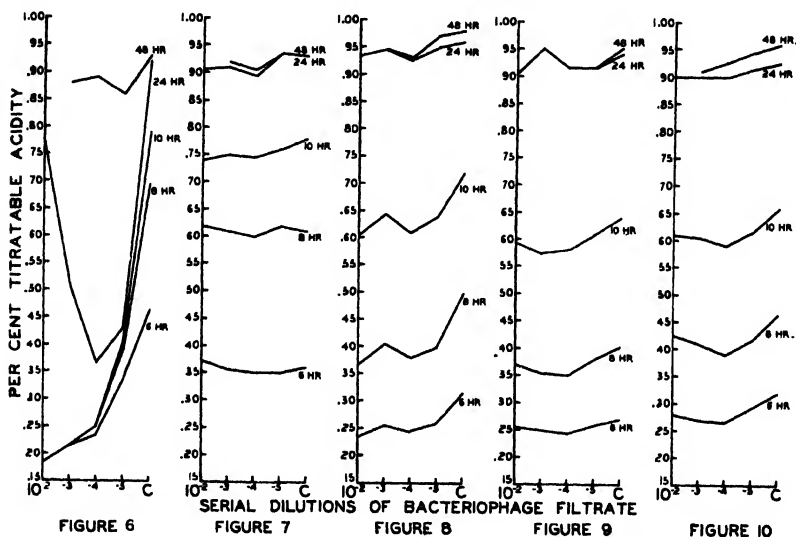
The steadily increasing resistance to bacteriophage of the newly developed strains of lactic acid organism became noticeable soon after the start of series 3. In series 3 and 4 (figs. 3 and 4) the culture which received the greatest concentrations of added bacteriophage filtrate still showed some inhibition in acid production as compared to the controls, especially at the 10-hour titration period, but in series 5 (fig. 5) the acidities followed very nearly a straight line across the graph, except for slight variations which could well be expected. The fact that the culture of series 5, which contained a serial dilution of 10^{-2} of bacteriophage filtrate, showed practically the same titratable acidity at each titration interval as the control indicates that the lactic streptococci of this strain had become well adapted to growth in the presence of active bacteriophage after only a relatively few transfers in milk to which bacteriophage filtrate had been added.

The somewhat lower acidities developed by each culture in series 3, 4 and 5 at the 6-, 8- and 10-hour titration periods as compared to the control of series 1, which was inoculated from an actively growing culture, can be attributed, in part at least, to the general "slowing down" effect on organisms in cultures that are not transferred frequently enough. The magnitude of this effect could have been determined in the present experiment had a control culture, with no added bacteriophage filtrate, been included with each experimental series. However, at the 24- and 48-hour titration intervals the titratable acidity of practically every culture in series 3, 4 and 5 was higher than the control culture of series 1.

Adaptability of strain no. 1 to the homologous bacteriophage. This experiment included five successive culture series. The general experimental procedure

was the same as that used in the experiments with strain H. P. In the studies with the no. 4 strain, only five flasks were used in each series because the titer of the bacteriophage filtrate active against this organism, as shown by preliminary observations, was not very high. To four of these flasks were added serial dilutions of the homologous bacteriophage filtrate ranging from 10^{-2} to 10^{-5} , inclusive, and the fifth served as a control.

The data from the titrations after 6, 8, 10, 24 and 48 hours of incubation of the milk cultures are presented in figures 6 through 10. The data from the 2- and 4-hour titrations were not graphed because they did not show any significant change in acidity.



FIGS. 6 through 10. Adaptability of strain no. 4 to the homologous bacteriophage. Amount of acidity developed at 30° C.

The data from series 1 (fig. 6) indicate that the titer of the bacteriophage filtrate used in this experiment was at least as high as 10^{-5} . It cannot be stated conclusively whether or not it was higher than this, because a culture containing a serial dilution of 10^{-6} of filtrate was not included in this experimental series. It is very evident, however, that the bacteriophage filtrate active against culture no. 4 was not nearly as potent as the one used in the studies with culture H. P. It will be noted further that the organisms in two of the cultures in series 1 had become fairly well adapted, sometime between the 10- and 24-hour titration periods, to growing in the presence of the bacteriophage to which they were initially susceptible. The titratable acidity readings of series 1 at the 24-hour interval are very significant, because they show that the organisms of this particular culture developed a resistance to the bacteriophage more quickly in the presence of a heavy inoculation of the bacteriophage filtrate than they did when

a lesser quantity was added. The earlier recovery was particularly noticeable in the cultures to which serial dilutions of 10^{-2} and 10^{-3} of bacteriophage filtrate were added.

The results show that culture no. 4 had attained its maximum adaptability for growth in the presence of the homologous bacteriophage by the end of series 2 (fig. 7). The somewhat lower acidities developed by each culture in series 3, 4 and 5 (figs. 8, 9 and 10) at the 6-, 8- and 10-hour titration periods as compared to the control of series 1 and all cultures in series 2 can be attributed, at least partially, to the general "slowing down" effect on organisms in cultures that are not transferred frequently enough. It will be noted, however, that there were no great differences in the titratable acidities of any of the cultures in series 2, 3, 4 and 5 at the 24- or 48-hour titration intervals.

TABLE 1
Comparison of adapted and non-adapted cultures after storage at 0° C.

Hr. incubated at 30° C.	Titratable acidity (% lactic acid)					
	Culture—H. P.			Culture—no. 4		
	Adapted	Non-adapted	Control	Adapted	Non-adapted	Control
After 1 month						
2	0.190	0.185	0.180	0.180	0.185	0.180
4	0.255	0.185	0.220	0.240	0.220	0.225
6	0.390	0.185	0.330	0.400	0.235	0.345
8	0.585	0.185	0.535	0.620	0.235	0.555
10	0.705	0.185	0.670	0.725	0.250	0.695
24	0.820	0.375	0.800	0.815	0.445	0.825
48	0.820	0.730	0.805	0.830	0.830	0.860
After 4.5 months						
2	0.175	0.180	0.180	0.180	0.180	0.180
4	0.195	0.195	0.230	0.245	0.250	0.250
6	0.195	0.195	0.345	0.410	0.370	0.420
8	0.195	0.195	0.600	0.590	0.390	0.590
10	0.200	0.195	0.685	0.685	0.405	0.685
24	0.520	0.250	0.740	0.795	0.430	0.810
48	0.760	0.720	0.760	0.815	0.610	0.830

The ability of adapted strains of organisms to retain their adaptability to a specific race of bacteriophage. Transfers were made of the adapted cultures at intervals of 10 to 14 days. Except for the time during which newly-inoculated cultures were being incubated, the cultures were stored in the refrigerator at 0° C. Approximately one month after the completion of the original adaptation studies, each of the "adapted" cultures was compared with a normal culture of the same strain to determine if the acquired characteristic was temporary, or whether it persisted after repeated transfers to new milk. The procedure for this test was as follows: One milliliter of the adapted culture was added to a flask containing 100 ml. of autoclaved milk. Also, 1 ml. of a normal culture of the same strain of organism was added to each of two similar flasks of milk. One milliliter of bacteriophage filtrate active against the strain of or-

ganism then was added to the flask containing the adapted inoculum, and the same amount of filtrate was added to one of the other flasks. The third flask of milk served as a control. The cultures were incubated at 30° C. Titratable acidity determinations were made after 2, 4, 6, 8, 10, 24 and 48 hours of incubation. The results of these determinations are presented in table 1. The data indicate that both the adapted H. P. culture and the adapted no. 4 culture retained their ability to resist attack by the bacteriophage active against the original cultures of the same strains of organisms. The same type of determination was made on these cultures after 4.5 months of storage at 0° C. The results of this experiment also are presented in table 1. The results of this trial

TABLE 2
*Comparison of adapted and non-adapted cultures after approximately
4.5 months of storage at 0° C.*

Hr. incu- cubated at 30° C	Titratable acidity (% lactic acid)					
	Culture—H. P.			Culture—no. 4		
	Adapted	Non- adapted	Control	Adapted	Non- adapted	Control
2	0.175	0.180	0.180	0.180	0.180	0.180
4	0.195	0.195	0.230	0.245	0.250	0.250
6	0.195	0.195	0.345	0.410	0.370	0.420
8	0.195	0.195	0.600	0.590	0.390	0.590
10	0.200	0.195	0.685	0.685	0.405	0.685
24	0.520	0.250	0.740	0.795	0.430	0.810
48	0.760	0.720	0.760	0.815	0.610	0.830

show that the adapted H. P. culture had almost completely lost its previously-acquired resistance to the homologous bacteriophage. The only indication that it had retained a part of its acquired resistance was the fact that the titratable acidity of the adapted culture was markedly above the acidity of the normal culture at the 24-hour titration interval. The adapted no. 4 culture developed acid just as rapidly as the control culture, which contained no added bacteriophage; therefore, it must be concluded that it had retained its acquired resistance to the bacteriophage active against it even after 4.5 months.

These data indicate that one cannot predict accurately how long adapted cultures of lactic acid streptococci will retain the resistance that they acquire when grown in the presence of a homologous bacteriophage. The limited data available show that the degree of retention of this acquired characteristic will vary between different strains of organisms.

SUMMARY

In the present study, experiments were conducted to determine if single-strain cultures of lactic-acid streptococci could become adapted to grow in the presence of homologous bacteriophage, and if so, how long the acquired characteristic would persist.

The data show that single-strain cultures of lactic streptococci will acquire

an adaptation for growth in the presence of homologous bacteriophage. The length of time during which this acquired characteristic will persist varies with the strain of organism.

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EFFECT OF HIGH-TEMPERATURE SHORT-TIME HEAT TREATMENTS ON SOME PROPERTIES OF MILK. I. INACTIVATION OF THE PHOSPHATASE ENZYME¹

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Since the development of the phosphatase test by Kay and Graham (8) and its subsequent modifications, several studies have been made to determine the suitability of the test for detecting irregularities in the pasteurization of milk and other dairy products. The literature on this phase of the phosphatase test up to 1939 has been thoroughly reviewed by Burgwald (2). The reported studies showed that all raw milk contains phosphatase, that the thermal resistance of phosphatase is greater than that of pathogens, and that the test is sufficiently sensitive to detect important variations in pasteurizing conditions on the basis of the residual phosphatase activity. Other work has been directed toward improvement of the phosphatase test to make it more sensitive, more rapid, and more quantitative (3, 5, 13, 15).

Several investigators (6, 7, 11, 14, 17) have reported on the time-temperature relationships necessary to inactivate the phosphatase enzyme in milk. The results obtained differ considerably, probably because of the variety of methods used for heating the milk and for testing the phosphatase activity, as well as differences in accepted standards of what constitutes satisfactory destruction of the enzyme. Holland and Dahlberg (6) stated that most of the discrepancies probably could be accounted for by the variations in the length of time required to heat to and cool from the temperatures at which they are holding. They did not, however, present any data to show the effect of various rates of heating. Later Lythgoe (9) commented that if milk is heated very quickly the time of inactivation of phosphatase necessarily may be longer than if milk is heated more slowly, but no data were given.

Previous experimental work has shown that at temperatures above 140° F. phosphatase destruction proceeds with sufficient rapidity to make time of heating to temperature extremely important and, as the temperature to which milk is heated becomes higher, the cumulative effects of heat in reaching the temperature become progressively more important. Using a heating time of 35-40 seconds, Prucha and Corbett (11) found the phosphatase to be inactivated by an instantaneous exposure at 160° F. They used Scharer's (15) test, measuring the indophenol color with the Hahn and Tracy (5) photoelectric cell set-up, and placed the standard for satisfactory destruction of phosphatase at 0.8 p.p.m. phenol equivalent. Holland and Dahlberg (6) secured a negative phosphatase

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test by heating to 170° F. using a 10-second heating time above 140° F. and the Kay and Graham (8) test with the standard of 0.04 mg. phenol equivalent.

In June, 1946, an experimental unit built on Mallory (10) principles by the Illinois Creamery Supply Company, Chicago, Illinois, was installed in the research laboratory of the Dean Milk Company, Rockford, Illinois. With this equipment, it was possible to heat cold milk to any desired temperature well above the boiling point in 0.83 second.

Since the trend in design of continuous milk heating equipment is toward faster, more efficient heating with more precise control of flow rate and temperature, it would seem that higher temperatures and shorter time processing will result and more information will be necessary to set time-temperature relationships. It also was apparent that information should be secured on the effects of various rates of heating on the temperatures required to inactivate the phosphatase to provide a better basis for evaluating the results of other investigators in this field. The results of such a study could be used to form a basis for formulating a practical mathematical time-temperature solution for the inactivation of the phosphatase enzyme which would explain the results secured by any heating method. It also was decided to study seasonal variations in phosphatase content of milk and the distribution of the phosphatase enzyme in various milk fractions.

EXPERIMENTAL METHODS

All of the experimental work was done in the research laboratory of the Dean Milk Company. The milk used for this study was fresh Grade A raw milk as received at the plant from the company's patrons. The Mallory small-tube heat exchanger was used to process the milk when various times and temperatures required to inactivate the phosphatase enzyme were studied. This unit had a capacity of 80 gallons per hour. It was composed of five independently operating heating sections and two cooling sections. Milk was forced through the unit with an 80-gallon-per-hour Manton-Gaulin homogenizer used as a high pressure pump. A pressure of 800 to 1,000 lb. per square inch was required to force milk through the unit. Each heating section was composed of four 58.5-inch lengths of $\frac{1}{4}$ -inch O. D. stainless steel tubing, through which the milk flowed, surrounded by a larger pipe containing dry steam as the heating medium. The time required for the milk to flow through one heating or cooling section was calculated to be 0.83 second and the milk flowed through the unit at a calculated velocity of 23.6 feet per second. After the milk was heated to the desired temperature, it flowed through a copper coil of $\frac{3}{8}$ -inch O. D. immersed in a water bath held at the desired holding temperature. The coil was so constructed that milk could be removed after any desired length of time directly into test tubes which previously had been immersed in ice water. The temperatures were measured with a mercury-in-glass thermometer inserted in a mercury well which was placed in the line between the Mallory unit and the copper holding coil. In this study, the time required to heat to all maximum temperatures was 0.83 second.

The phosphatase activity in terms of phenol equivalent was measured by the

method of Sanders and Sager (13) with one modification; i.e., the color development buffer used was the one proposed by them (12) in a previous publication. One-hour incubation time at 37° C. and half-hour color development at room temperature were used. The indophenol was extracted with 10 ml. of buffered butyl alcohol and the transmission measured at 650 m μ , using a Coleman model 11 spectrophotometer and a 1.3-cm. square cuvette. Boiled milk controls were run with each series of determinations. The quantities of phenol, after consideration of the boiled controls, were read directly from a standard transmission-concentration curve prepared with known amounts of phenol. The results are expressed as micrograms of phenol per milliliter of milk or parts per million on a milk basis (not on the basis of the parts per million phenol in the butyl alcohol extract). This method was found to be sensitive and reproducible quantitatively; 0.05 per cent raw milk in boiled milk could be detected readily. Phenol added to pasteurized milk could be recovered satisfactorily.

The phosphatase tests were run approximately 24 hours after treatment of the milk.

RESULTS

Phosphatase activity in raw milk and distribution of the phosphatase enzyme in various milk fractions

Variations in the initial phosphatase concentration, if large, possibly would make some difference in the time and temperature required to inactivate the enzyme. Unfortunately, the phosphatase contents of the raw milk in the initial stages of the study were not accurately determined and only those values determined since February, 1947, will be given. The initial phosphatase content was so high that insufficient di-sodium phenyl phosphate was used and this was not recognized until the values were checked by diluting the raw milk samples with boiled milk to bring the phenol concentrations within the range of the standard curve concentration. Since the kinetics of the reaction between the enzyme and the substrate in the test itself is reported to be first order, the initial concentration will have some effect on the rate of the reaction when a standard length of time of incubation is used. For this reason all raw samples were diluted with boiled milk to make the final phenol concentration fall within a rather narrow range (range of standard curve, 0–20 γ phenol), and the concentrations then were calculated on the raw milk basis.

The phosphatase values of ten lots of raw milk from February 20 to July 9, 1947, ranged from 1,920–3,000 p.p.m. with an average of 2,230 p.p.m. These values are of the same order of magnitude as the value reported by Sanders and Sager (13) for whole milk and are considerably higher than those secured by previous investigators.

To secure some idea of the distribution of phosphatase in milk, raw milk was separated into cream and skim milk. The raw cream was churned into butter and the raw buttermilk drained off. The butter was melted at 110° F. and centrifuged to secure butter oil devoid of phospholipid material. The pH of the raw skim milk was adjusted to pH 4.6 with 0.1 N hydrochloric acid and the casein

was filtered off. The filtrate was neutralized to pH 6.7 with 0.1 *N* sodium hydroxide and the phosphatase activities of all fractions were determined. Each sample was diluted with boiled milk before running the phosphatase test to bring the final phenol concentrations in range of the standard curve. The results are calculated on the product basis by weight and reported as p.p.m. phenol equivalent. The results (fig. 1) indicate that the enzyme probably is concentrated at the fat-serum interface, perhaps in a manner similar to agglutinin, since butter oil showed no phosphatase activity and the buttermilk showed an activity approximately ten times that of skim milk. This is in agreement with the observations of Kay and Graham (7). It should be observed, too, that the phosphatase activity of the raw milk was recovered quantitatively in the skim milk and cream.

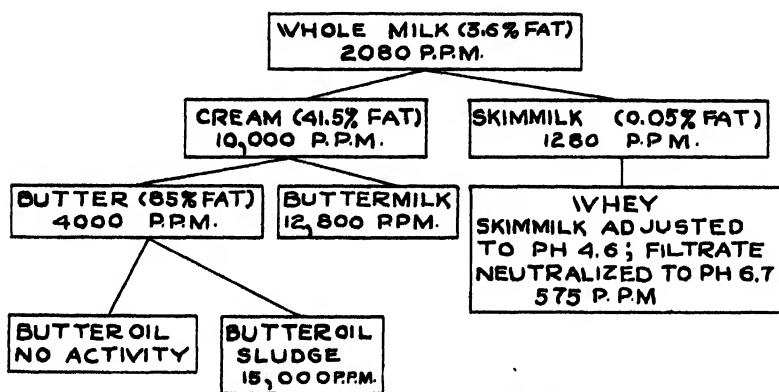


FIG. 1. Distribution of phosphatase in the various milk fractions. Phenol equivalent on the product basis by weight (p.p.m.).

The separation temperature in this case was 86° F. Figure 1 also shows that there is sufficient phosphatase activity in the various milk fractions to permit use of the phosphatase test to determine whether or not most dairy products have been properly pasteurized. The only exception appears to be butter oil.

Since the phosphatase enzyme apparently was concentrated at the fat-serum interface, a study of the effect of separating temperature on the distribution of the enzyme between cream and skim milk was thought desirable. If held in a similar manner as agglutinin, it was thought that an increase in the separating temperature would result in release of the enzyme from fat and the skim milk upon separation would show more enzyme activity. Consequently three lots of raw milk from the same milk source were separated, one at 80° F., another at 100° F., and a third at 120° F. Each lot of cream was standardized to 39.5 ± 0.5 per cent fat with skim milk from the same separation, and all samples (milk, cream and skim milk) were tested for phosphatase activity. The results are recorded in table 1.

Although some reduction in phosphatase activity of the cream resulted from increasing the separating temperature, a corresponding increase in the skim milk

fraction was not observed, which suggests the possibility that some phosphatase activity may be lost in the separator slime as the separating temperature is increased. It commonly is observed that as the temperature of separation and centrifugal clarification increases, the quantity of slime also increases, which may account for the losses in phosphatase activity observed in the cream (table 1). Actual test of the separator slime in another experiment showed it to contain considerable phosphatase activity. The reduced phosphatase activity also may be due to slight inactivation by the higher temperatures, but this is not borne out by data to be presented later. At any rate, the phosphatase apparently is held to fat more tenaciously than is agglutinin. It should not be inferred, however, that the phosphatase is entirely on the fat.

TABLE 1

Effect of separating temperature on the distribution of phosphatase in the cream and skim milk fractions

Separating temp. (°F.)	Phosphatase activity (p.p.m. phenol)			
	Milk	Cream	Skim milk	Reconstituted milk
80	2,130	10,400	1,370	2,190
100	2,100	9,400	1,360	2,090
120	2,090	8,600	1,340	2,000

Selection of phenol concentration standard for satisfactory inactivation of the phosphatase enzyme

The distribution of phosphatase activity in cream and skim milk brought up an interesting thought with respect to phenol standards for satisfactory destruction of the enzyme. If one were to pasteurize milk and select the 4 p.p.m. phenol equivalent standard as proposed by Sanders and Sager (13), cream separated from this milk would, it was thought, show a positive phosphatase test. Positive phosphatase tests on cream separated from pasteurized milk have been reported by Scharer (16), using his rapid method.

Milk was heated in the steam jacketed hot well in such a way as to give phenol equivalents greater than 4 p.p.m. and less than 4 p.p.m. and the resulting milk cooled and held two hours. The lots of milk then were heated to 80° F. and separated into 32 per cent cream and skim milk, and the phosphatase activities of the milk, cream, and skim were determined to see if the distribution of the enzyme would be essentially the same as when unheated milk was separated. When milk with a phosphatase activity of 7.5 p.p.m. was separated, the cream and skim milk showed phosphatase activities of 12.8 and 5.4 p.p.m., respectively. When milk with a phosphatase activity of 3.3 p.p.m. was separated, the phosphatase of the cream and skim milk separated from this milk were found to be 4.9 and 2.2 p.p.m., respectively.

While the cream showed much higher phosphatase activity than the milk, the differences were less than when cream and skim milk were separated from raw milk. The cream showed a positive phosphatase test even when the milk was

properly pasteurized according to the Sanders and Sager (13) standard. In another experiment in which milk was heated to give a phosphatase activity of 1.9 p.p.m. phenol equivalent and the resulting milk separated into 25 per cent cream, the cream showed a phosphatase activity of 2.8 p.p.m. phenol. Apparently this relationship would hold no matter what phenol concentration is selected unless the phosphatase were completely inactivated. However, the lower the phosphatase activity of the milk the smaller the spread between the phosphatase activity of milk and cream seems to be. Then too, values of less than 1.0 p.p.m. are not readily distinguishable by the test. To minimize the discrepancy between properly pasteurized milk and cream separated therefrom, keeping in mind the accuracy of the test, a value of 1.0 p.p.m. was adopted as the standard for satisfactory inactivation in this study. This standard requires temperatures higher, approximately 1-3° F., depending upon the corresponding holding time, than the 4 p.p.m. standard of Sanders and Sager (13). Gilcreas and O'Brien (4) indicated that higher temperatures than present pasteurization standards are necessary to kill *E. coli*, which may justify elevating the standards for the phosphatase test.

From a practical standpoint, it should be pointed out as a conclusion to this phase of the study that either the phenol standards must be varied to suit the various dairy products being pasteurized at constant time-temperature conditions or, if a single phenol standard is selected, more severe heat treatments will have to be administered to some products than others to call them properly pasteurized. The data of Sanders and Sager (14) verify this conclusion.

Kinetics of inactivation of phosphatase by heat treatment and time-temperature relationships

If the reaction is first order, as indicated by Van Bever and Straub (17), a plot of the log of the concentration against the time should yield a straight line. Milk was heated in the hot well to 143° F. and the relationship of time at constant temperature (143° F.) to the concentration is given in figure 2 for skim milk and whole milk. The reaction is not strictly first order but possibly a pseudo-first order. Much the same results were secured at higher temperatures using the Mallory unit to heat and the holding accomplished with the coil described previously. These data are given in table 2.

Van Bever and Straub (17) have derived a mathematical expression from the first order reaction rate formula and Arrhenius equation with which they should be able to predict the residual phenol concentration for any time-temperature cycle if these relationships hold. From the results shown in figure 2, this did not seem to be readily possible because the reaction did not appear to be strictly first order. As the destruction of phosphatase proceeded, the rate of destruction at constant temperature became slower with time than would be indicated by straight semi-log relationship. This difference possibly could be explained by differences in the method of determination of the phosphatase used in this investigation and that used by Van Bever and Straub (17), and differences in the range

of concentrations studied. They found the relationship to hold during up to 96 per cent destruction of the original phosphatase content.

The thermal processing work of Ball (1) suggested an approach to the problem through mathematical solution. Several investigators (6, 8, 14, 17) have

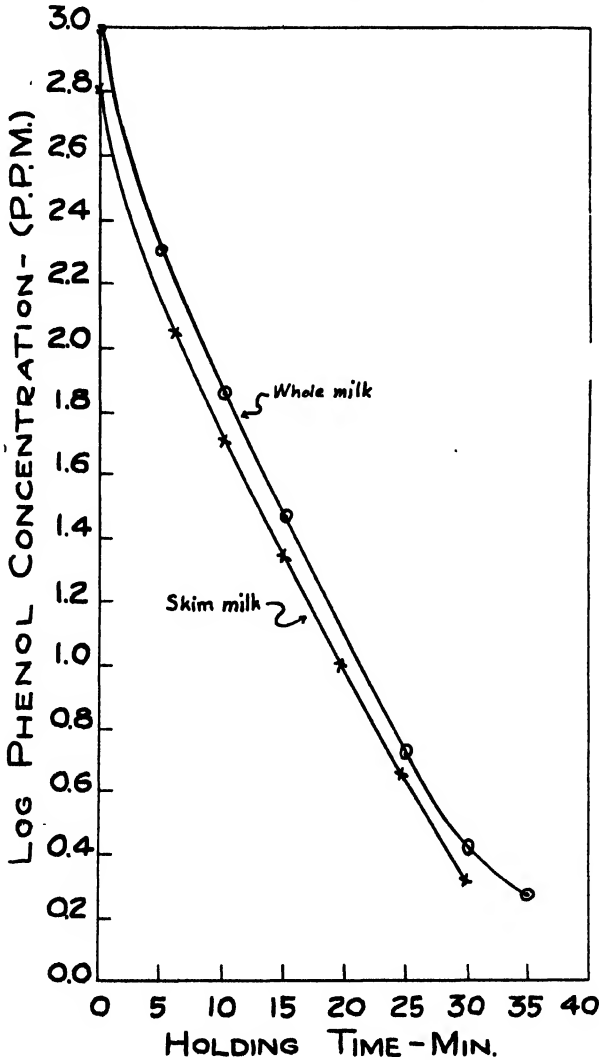


Fig. 2. Change in phosphatase concentration with time at 143° F.

shown that a plot of the temperature vs. the log time for inactivation at that temperature yields a straight line. Using 0.8-second heating time and holding in the coil described previously, the times required at various temperatures from 143 to

185° F. to reduce the phosphatase concentration to 1 p.p.m. phenol equivalent were determined. The results plotted in figure 3 show the same relationship secured by previous investigators. The formula for the curve shown in figure 3 is: $T = 174 - 9 \log t$, where T is the temperature in °F. and t is the time in seconds required at temperature T to inactivate the phosphatase. From this relationship it would seem that a practical solution for time-temperature cycles could

TABLE 2
*Effect of time and temperature on the phosphatase activity in whole milk
(0.8 sec. required to heat to all temperatures)*

Temperature	Time	Phenol
(°F.)	(sec.)	(p.p.m.)
Raw milk		2,180
150	306	9.3
	361	2.0
	420	1.0
155	2	1,265
	32	67
	64	15
	92	1.6
	112	0.9
Raw milk		2,020
160	2	340
	13	6.0
	22	1.4
	32	0.6
165	2	55
	7	
	10	1.0
	15	0.6
Raw milk		3,000
159	.03	2,480
165	.03	1,580
170	.03	855
175	.03	137
180	.03	4.8
185	.03	1.0
171	1	11.8
172	1	6.0
173	1	1.6
174	1	1.0
175	1	0.4

be developed if one considered a single final phenol concentration (1 p.p.m.). The destructive effect (hereafter called the D value) of any temperature for each second hold in inactivation of the phosphatase (1 p.p.m.) can be given as:

$D = \log^{-1}_{10} \frac{(T - 174)}{9}$ and the summation of the D values times the time at the corresponding temperatures must be 1 or greater to insure satisfactory inactivation of the phosphatase.

Using this formula it can be shown that it would require 6 hours to inactivate the enzyme at 135° F. This was verified experimentally and this temperature was selected as a starting point for calculating the D values listed in table 3. The third column in table 3 shows the cumulative D values at any temperature as-

suming straight line heating and 1-second per degree F. heating rate. It should be stated that this was arranged for convenience in calculating cumulative D values for experimental data secured later using constant heating rates. But, according to this method, one could calculate a cumulative D value by plotting the individual D values corresponding to the temperatures used against the time in seconds. The area under the resulting curve would be the cumulative D value and this should be 1 or greater for any time-temperature cycle if the phosphatase is inactivated.

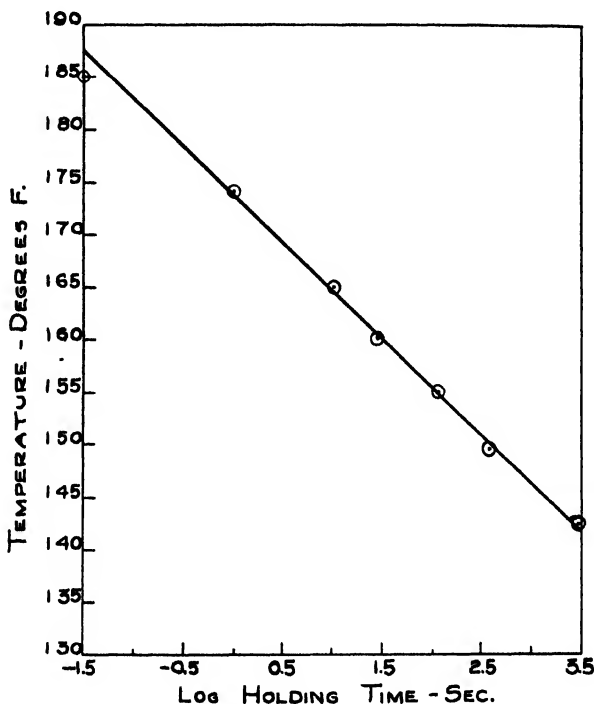


FIG. 3. Time-temperature relationship for the inactivation of the phosphatase enzyme in milk.

Effect of heating rate on temperature necessary to inactivate the phosphatase enzyme in milk

To secure some evidence on whether or not the mathematical solution has practical and proved application and to find the relationship between hot well heating at various rates and Mallory heating, the following experiments were performed.

Raw whole milk was heated in the hot wells by varying the quantities of milk and steam so that various line (constant change in temperature with time) heating and various heating rates from 2 to 20° F. per minute were secured. Samples were obtained at various temperatures and cooled rapidly in ice water as the

milk was being heated; the temperature at which the phenol concentration became 1 p.p.m. was determined. The results are listed in table 4, along with

TABLE 3

Comparative destructive effect of heat on the phosphatase enzyme activity in milk

Temperature (°F.)	D value/sec. hold	Cumulative D value (1 sec./°F.) (Straight line heating)
135	0.000046	0.000046
136	0.000059	0.000105
137	0.000077	0.000182
138	0.0001000	0.000282
139	0.000129	0.000411
140	0.000167	0.000578
141	0.000215	0.000793
142	0.000277	0.001070
143	0.000358	0.001428
144	0.000462	0.001890
145	0.000597	0.002487
146	0.000772	0.003259
147	0.00100	0.00426
148	0.00129	0.00555
149	0.00167	0.00722
150	0.00215	0.00937
151	0.00277	0.01214
152	0.00358	0.01572
153	0.00462	0.02034
154	0.00597	0.02631
155	0.00772	0.03403
156	0.0100	0.0441
157	0.0129	0.0570
158	0.0167	0.0737
159	0.0215	0.0952
160	0.0277	0.1229
161	0.0358	0.1587
162	0.0462	0.2049
163	0.0597	0.2646
164	0.0772	0.3418
165	0.100	0.442
166	0.129	0.571
167	0.167	0.738
168	0.215	0.953
169	0.277	1.230
170	0.358	1.588
171	0.462	2.050
172	0.597	2.647
173	0.772	3.419
174	1.00	4.42
175	1.29	5.71
176	1.67	7.38
177	2.15	9.53
178	2.77	12.30
179	3.58	15.88
180	4.62	20.50
181	5.97	26.47
182	7.72	34.19
183	10.0	44.2
184	12.9	57.1
185	16.7	73.8

data secured by Mallory heating and holding. The corresponding calculated cumulative D values also are shown. The dates on which the individual experi-

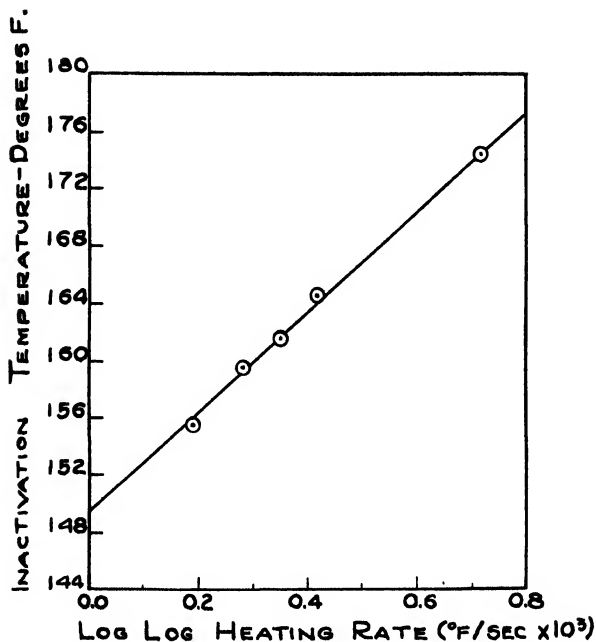


FIG. 4. Effect of heating rate on the temperature required to inactivate the phosphatase enzyme in milk.

ments were performed are given to indicate any effect season may have had on time-temperature relationships.

As a sample calculation of the cumulative *D* values, consider the first set of values given in table 4. The heating rate was 0.035° F. per second or the milk was held 28.6 seconds per °F. as it was heated from 135 to 155° F. The cumulative *D* value at 155° F. is 0.03403 if held only 1 second per °F. Therefore, by

TABLE 4

Effect of heating rate on the temperatures required to inactivate the phosphatase enzyme in milk (1.0 p.p.m. phenol equivalent)

Type of heating	Date	Heating rate (°F./sec.)	Temp. (°F.)	Holding time (sec.)	<i>D</i> value
Hot well	10/ 1/46	0.035	155	1	0.98
	10/21/46	0.085	159	1	1.14
	9/17/46	0.167	161	1	0.99
	9/17/46	0.390	164	1	0.95
	10/ 1/46	0.178	143	3,000	1.08
	11/27/46	0.177	152	270	1.05
Mallory	8/ 9/46	137	174	1	1.03
	3/28/47	150	185	0.03	0.99
	5/14/47	120	165	10	1.00
	5/14/47	108	150	420	0.91

the time the milk reached 155° F., the D value equals 28.6×0.03403 or 0.97. To this must be added the D value \times time at 155° F. = $0.0077 \times 1 = 0.0077$. Thus, the cumulative D value would be 0.98.

Figure 4 shows the relationship between heating rate and temperature necessary to inactivate phosphatase with one second hold at the top temperature. The temperature of inactivation is a log log function of the heating rate. Extending the curve to 0 log log heating rate ($^{\circ}\text{F./sec.} \times 10^3$) indicates a temperature of 149° F. to be sufficient to inactivate the phosphatase. Theoretical calculations from the D values indicate a temperature of 150° F., which is fairly good agreement.

Apparently the mathematical solution is essentially satisfactory considering the constancy of the cumulative D values. If one considers an error of 1° F. in top temperature as being possible, the cumulative D values may vary approximately 0.2 and all values listed in table 4 are within this limit.

SUMMARY

Information was secured on the phosphatase activity of raw milk and the distribution of phosphatase in various milk fractions. The phosphatase is believed to be located to a large extent but not entirely at the fat-serum interface. Separation temperatures up to 120° F. did not appreciably change the distribution of the phosphatase in the cream and skim milk fractions. Separation of heated milks possessing various phosphatase activities showed that the lower the phosphatase activity in the milk the less the difference in the phosphatase activity in the cream over that in the milk. A standard of 1 p.p.m. phenol was selected as satisfactory destruction considering the limits of accuracy of the test and the smallest increase from a practical standpoint in phosphatase activity in cream over that in milk from which this cream was separated.

A study was made of the kinetics of phosphatase inactivation by heat, and time-temperature relationships for the inactivation of phosphatase are given over the temperature range of 143 to 185° F.

A mathematical solution for time-temperature cycles is given which takes into consideration accumulative effects of heating to the holding temperature in reducing the phosphatase activity to 1 p.p.m. phenol. Data secured with various rates of heating indicated that the mathematical solution is satisfactory for practical use in determining time and temperature necessary to give a negative phosphatase test in milk with various heating methods. It follows that, if the phosphatase test is used as the standard for adequate pasteurization, this mathematical solution can be applied to determine the proper time-temperature conditions.

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EFFECT OF HIGH-TEMPERATURE SHORT-TIME HEAT TREATMENTS ON SOME PROPERTIES OF MILK. II. INACTIVATION OF THE LIPASE ENZYME¹

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Although there was considerable disagreement in the early literature concerning the presence of lipase enzyme in milk, the work of Rice and Markley (13) and Palmer (11) in 1922 showed lipase to be a normal constituent of cow's milk.

This enzyme is capable of splitting the glyceride structure of milk fat, liberating free fatty acids. The low molecular weight fatty acids thus formed, such as butyric and caproic acids, give the milk its characteristic rancid odor and flavor. It appears as though there may be more than one lipase present in milk (6). In general, two types of lipolysis now are recognized; one is the spontaneous and the other induced lipolysis. This study deals with the induced type of lipolysis. In induced lipolysis, the system must be "activated", possibly by changing the normal adsorbed layer surrounding the fat globules, thus making the fat more susceptible to lipase action. This can be accomplished by shaking (9), by homogenization at temperatures below 130° F. (2), and by temperature treatment between 40–80° F. (8). In this study, lipolysis was induced by homogenization at 105° F.

The lipase enzyme is heat labile. While it is not known whether both types of lipolysis respond similarly to heat treatment, the indications are that both types are destroyed under standard pasteurization conditions using the holder method. Tarassuk (14) found that 130° F. for 30 minutes would prevent the spontaneous type of lipolysis. Dörner and Widmer (2) and Halloran and Trout (5) found that pasteurization before homogenization stopped this type of induced lipolysis. Doan and Minster (1) have shown that rancidity invariably was present 24 hours after homogenization unless the milk had been previously heated to 150° F. without holding. Gould and Trout (4) found no change in fat constants or acid degree of milk fat when milk was pasteurized at 145° F. for 30 minutes and homogenized at a pressure of 1,500 lb. per square inch. In their experiments, homogenization of raw milk caused an average increase of 1,625 per cent in fat acidity during the first 24 hours of storage at 35–40° F. In an experiment where milk was heated to 140, 150, 160 and 170° F. without holding and the milk subsequently made into sweetened condensed milk, Rice (12) found that only the milk preheated to 140° F. developed rancid flavor after 8 months of storage at room temperature. When milk was heated in the

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presence of sugar, a temperature of 180° F. was necessary to prevent this defect. Palmer (11) stated that heating milk to 75° C. (167° F.) for a few minutes would stop rancid and bitter flavor development. In these studies the heating times were not given.

The present study was designed to secure more information on the holding time of temperatures from 145 to 185° F. which is necessary to prevent the lipolysis induced by homogenization of raw milk, with extremely short heating and cooling times.

EXPERIMENTAL METHODS

The small-tube Mallory heat exchanger and the copper holding coil described previously (7) were used to process the milk. Raw whole milk was heated to 105° F. in the steam-jacketed hot well. The milk then was homogenized at a pressure of 1,500 lb. per square inch and pumped through the heat exchanger by means of the homogenizer acting also as a high pressure pump. Within 5 seconds after homogenization, the milk was heated to the desired holding temperature. After the desired holding times had elapsed, samples were drawn out of the holding coil into test tubes immersed in ice water. All samples were stored 44-48 hours at 40° F. before examination for rancid flavor and other properties.

Preliminary experiments were run to ascertain the most applicable methods for determining the extent of lipase action. Gould and Trout (4) stated that fat acidity was the most reliable criterion, but difficulty was experienced by the present authors in churning the homogenized milk and extraction methods were not considered reliable. Titratable acidity and pH measurements of the milk did not prove as sensitive as organoleptic examination. Doan and Minster (1), Halloran and Trout (5), and Tarassuk and Henderson (15) have used surface tension changes as an index of extent of rancidity. The usual surface tension of the milk used in this study was about 44 dynes/cm. at 20° C. and the surface tension of butyric acid is about 26 dynes/cm. at 20° C. Preliminary experiments indicated that surface tension changes were sufficient when rancidity developed to use this as a measure of lipase activity. These measurements always were supplemented with organoleptic examination.

Surface tension was measured with a du Noy tensiometer at 20-21° C. Measurements were made in a constant temperature (21° C.) room after the samples had been brought to a temperature of 20° C. in a water bath. The tensiometer was standardized to absolute units with materials of known surface tension and the results are reported in dynes per centimeter. Each reported result is an average of at least three determinations whose maximum variations were within ± 0.5 dynes/cm. of the average. Organoleptic examinations were made on the same samples. The samples were scored by at least three competent judges for rancid flavor, and the results reported on the basis of 0 for no rancid flavor, ? for questionable, 1 for definite, 2 for pronounced, and 3 for very pronounced rancidity.

EXPERIMENTAL RESULTS

Raw milk was heated in 0.83 second to various temperatures within 5 seconds after homogenization, held for various lengths of time and then rapidly cooled. The changes in flavor and surface tension of the milk after 44-48 hours of storage at 40° F. are shown in table 1. Four different lots of milk were used to secure the data listed. No rancid flavor developed in any of the lots of raw unhomogenized milk in the 44 to 48-hour storage period at 40° F., and the surface tension of these lots of milk varied from 44.0 dynes/cm. to 44.7 dynes.

TABLE 1

Effect of heating milk on lipolytic action induced by homogenization of raw milk at 105° F.

Lot no.	Sample no.	Temperature	Time held	Rancid flavor ^a	Surface tension ^a
		(°F.)	(sec.)		(dynes/cm. 20° C.)
1	1a	177	0.03	2	42.0
	b	180	0.03	2	41.7
	c	184	0.03	†	42.4
	d	186	0.03	0	45.4
1	3a	160	2	2	39.9
	b	163.5	2	2	41.9
	c	167	2	0	44.0
	d	169	2	0	44.8
2	2a	164	1	3	39.5
	b	167	1	2	39.5
	c	170	1	†	43.5
	d	173	1	0	44.5
3	4a	160	2	2	40.6
	b	160	6	1	42.6
	c	160	10	0	45.4
	d	160	14	0	45.6
3	5a	155	2	3	39.3
	b	155	12	1	42.1
	c	155	22	0	44.7
	d	155	32	0	45.0
4	6a	150	33	2	40.8
	c	150	60	†	43.2
	d	150	120	0	44.0
2	7a	145	200	1	41.5
	b	145	300	†	43.2
	c	145	400	†	43.8
	d	145	500	0	44.0

^a After storage at 40° F. for 44-48 hours.

Very pronounced rancid flavor was present in each of the lots of milk which were homogenized raw at 105° F. and stored 44-48 hours at 40° F. The surface tension of the raw homogenized milk after storage ranged from 35.0 dynes/cm. to 37.1 dynes/cm.

When milk was heated sufficiently to prevent lipolytic activity, as indicated by the organoleptic examination, the surface tension of the heated milk was essentially the same as that of raw unhomogenized milk. Decreases in surface tension accompanied the rancid flavor development on those samples which were

not heated sufficiently to stop lipase activity. This is in agreement with the work of others (1, 5, 15).

From the data in table 1 and consideration of other trials in which flavor changes only were observed, the time-temperature heat treatment relationships given in figure 1 are believed to be ample for prevention of lipolytic action induced by homogenization of raw milk.

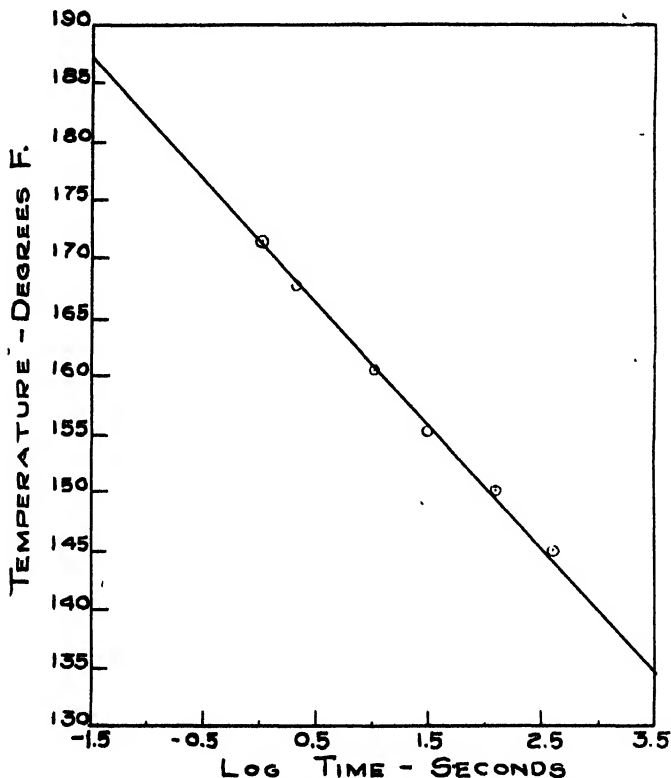


FIG. 1. Time-temperature relationship for the inactivation of the lipase enzyme in milk.

The data plotted in figure 1 show temperature to be a straight line function with log time, as was the case with the inactivation of the phosphatase (7). At 185° F. the time of inactivation is the same for both phosphatase and lipase, but the slope of the lipase curve is greater than that of the phosphatase; i.e., as the temperature is lowered, lipase is inactivated with progressively less time of exposure than is the phosphatase.

If one extrapolates the curve in figure 1 to 30 minutes holding time, a temperature of 137° F. is indicated as necessary to prevent this induced type of lipolysis. Krukovsky and Sharp (10) found that a temperature of 135° F. for 30 minutes was required to prevent lipolysis induced by the warming and cool-

ing procedure of Krukovsky and Herrington (8); Tarassuk (14) found 130° F. for 30 minutes was sufficient to prevent the spontaneous type of lipolysis. Either there is a difference in the heat treatment necessary to inactivate the enzyme in the two systems, or perhaps the discrepancy could be explained by the differences in methods used to follow lipase activity or by the differences in the rates of heating to and cooling from the holding temperatures employed.

Effect of Rate of Heating

These samples were heated to the temperatures indicated within 5 seconds after homogenization. In unheated samples rancid flavor developed within 5 to 10 minutes after homogenization. With a slow rate of heating (5° F. per minute), such as would be the case if a commercial pasteurizing vat were used, the rancid flavor developed before the holding temperature was reached. If homogenization were done after heating, one could demonstrate the importance of heating rate. In one experiment, milk was heated at a constant rate of approximately 5° F. per minute and portions were homogenized as the milk was being heated. These were cooled immediately over the surface cooler and stored at 40° F. A temperature of 142° F. was sufficient to prevent the development of the rancid flavor for at least 48 hours. The results of previous experiments listed in table 1 show that a temperature of 185° F. without holding is necessary to prevent lipolysis when the heating is accomplished in 5 seconds. The effect of heat is accumulative and the temperature and holding time necessary to inactivate the enzyme will be determined by how rapidly one heats to and cools from the temperature at which the milk is being held.

Influence of Copper on Lipolysis Induced by Homogenization

Herrington and Krukovsky (6) found that additions of 0.2–0.4 p.p.m. copper reduced lipolysis almost 20 per cent. Krukovsky and Sharp (10) showed that in the absence of dissolved oxygen, copper in concentration of 2–8 p.p.m. had almost no inhibiting effect on lipolysis induced by temperature manipulation (8). Gould's (3) results showed copper to have no significant effect on the extent of lipolysis in raw milk induced by homogenization. In this study, holding of the milk at various temperatures was accomplished by use of a copper holding coil, so the milk possibly was contaminated with copper. The previous results secured in this study might not be truly representative of the effect of heat treatment alone, but might be due to the combined effects of heat and copper.

Accordingly, raw milk was heated (105° F.), homogenized, and immediately heated to 145° F. with the Mallory, as in previous experiments. The milk was collected in test tubes and immersed in a constant temperature (145° F.) bath, held for various times and cooled in ice water. Flavor and surface tension determinations were made after 44 hours of storage at 40° F. The results, as well as those secured by the copper coil holding method, are given in table 2. These results indicate that there was no appreciable difference in degree of lipolysis whether milk was held in the copper holding coil or in glass test tubes immersed in water bath. Similar results were secured at 153° F. It was found

in one trial that milk which was held in the copper coil for 10 minutes at 145° F. contained 8.5 p.p.m. copper. It is believed that this would be above the maximum amount of copper which would be present in any sample in the study because the coil had not been used for quite some time and the accumulated copper oxide was not thoroughly removed previous to the trial. The control milk not exposed to the coil contained 0.11 p.p.m. Even the 8.5 p.p.m. copper secured under the most drastic treatment is below the level (10 p.p.m.) which Gould (3) found to have no effect on lipolysis.

TABLE 2

Effect of heating homogenized raw milk to 145° F. and holding various lengths of time in test tubes vs. holding in copper holding coil on the extent of lipolysis after 44 hours of storage at 40° F.

Samples ^a	Time (sec.)	dynes/cm. (20° C.)	Rancid flavor
1	200	41.5	1.0
1a	200	43.7	1.0
2	300	43.2	?
2a	300	43.9	1.0
3	400	43.8	?
3a	400	44.4	?
4	500	44.0	0
4a	500	44.5	0
Raw	not heated	37.1	3
Raw a	" "	37.9	3

^a Sample numbers followed by a were held in glass tubes; others were held in copper coil.

SUMMARY

The holding times at various temperatures from 145–185° F. required to prevent lipolysis induced by homogenization of raw milk were determined. A semi-log relationship of temperature with time was observed. At 145° F., approximately one-third the time was required to inactivate the lipase as was required to inactivate the phosphatase (7), but at 185° F. the same time was required to inactivate both enzymes.

Added copper had no noticeable effect on the time-temperature relationships for inactivation of lipase under the conditions of these experiments.

The time required at any temperature to inactivate the lipase was found to vary with the rate of heating to and cooling from the holding temperature. When milk was heated at the rate of 5° F. per minute, instantaneous exposure at 142° F. was sufficient to inactivate the enzyme, while heating by means of the Mallory unit within 5 seconds required a temperature of 185° F. with instantaneous exposure.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. II. EFFECT OF PREPARTAL RATIONS UPON THE NITROGENOUS CONSTITUENTS¹

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The importance of colostrum in sustaining the health of neonatal dairy calves has received renewed emphasis in recent years, yet little is known concerning factors affecting the properties of this product. One phase of a study of the relation of preparturient dairy rations to the health of the cow, to the well-being of the newborn calf, and to the properties of colostrum included an investigation of the effect of level of protein intake on concentrations of nitrogenous constituents in the mammary secretions during the postpartum transition from colostrum to milk. This phase of the investigation is reported herein.

PROCEDURES

Feeding and management of experimental animals. Twenty pregnant heifers and cows were paired according to breed and to number and stage of gestation. The heifers included two pairs of Guernseys and one pair each of Holsteins, Ayrshires and Jerseys. The cows, all in their second gestation, included two pairs each of Holsteins and Ayrshires and one pair of Jerseys. All animals calved within the 5-month period from the middle of November, 1945, to the middle of April, 1946.

Seven weeks (average) before parturition, one cow of each pair was given a high-protein ration consisting of a concentrate mixture (approximately 25 per cent crude protein), Atlas sorgo silage and alfalfa hay. The other cow of each pair was fed a low-protein ration consisting of corn, Atlas sorgo silage and prairie hay. Silage and hay were fed in the ratio of 3:1 to the extent of the appetite of each animal, and concentrates were given at the rate of 10 lb. per 1,000 lb. body weight. The same quantity of concentrate was fed throughout the trial, the initial body weights being used as a base for establishing the level of feeding. Starting on the ninth day postpartum, all cows were changed to the regular herd ration consisting of alfalfa hay, sorghum silage and a concentrate mixture containing approximately 16 per cent crude protein.

Collection of samples and analytical procedures. The calves were not allowed to nurse. The mammary secretions were withdrawn as completely as possible by standard milking methods, either hand or machine, at approximately 12-hour intervals. The first collection was made as soon as possible after parturition, usually within 4 hours. The total mammary secretions removed at each milking were well mixed before sampling. Colostrum and milk from each cow were ana-

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lyzed separately for each of the first six milkings and as composites for the seventh and eighth, the fifteenth and sixteenth, and the twenty-seventh and twenty-eighth milkings. Samples that were not analyzed immediately after collection were stored in a refrigerator at approximately 4° C. for a period not exceeding 4 days.

Rowland's method (14) was used to determine the nitrogen distribution. The only modifications of the method were the omission of selenium oxychloride as catalyst in the digestion procedure and the use of smaller samples for analysis. The amount of sample was reduced for two reasons: first, colostrum has a higher protein content than milk, which was the product for which the method was designed; and second, a micro-Kjeldahl apparatus was used in the distillation. The quantities of reagents for digestion and distillation were adjusted accordingly. Albumin and globulin nitrogen were not separated but were computed together by subtracting the values for non-protein nitrogen from those of non-casein nitrogen. Percentages of total protein and of casein were calculated from values for total nitrogen, non-protein nitrogen and non-casein nitrogen. Corrections were not applied to adjust for the volumes occupied by the precipitates of protein and fat.

RESULTS

Although considerable variation among the cows was observed, colostrum and early milk from animals of both groups had a similar total protein content and distribution of the components (fig. 1). While total protein, casein and albumin-globulin values for cows receiving the low-protein ration tended to be slightly higher during the first few milkings than were the values for corresponding samples from the high-protein group, the differences at none of the various periods were significant (*t*-test, $P = 0.05$). Concentrations of non-protein nitrogen were greater in both colostrum and early milk from cows receiving the high-protein ration than in these secretions from cows fed the low-protein ration. The differences, however, were significant only in samples representing the fourth, the fifth, the sixth, the seventh and eighth, and the fifteenth and sixteenth milkings. There was no significant difference in non-protein nitrogen of milk collected from the two groups on the fourteenth day (twenty-seventh and twenty-eighth milkings), which was 6 days after all cows had been changed to the regular herd ration.

Concentrations of the protein components tended to decrease logarithmically during the first four or five milkings of the transition period, the rate of change being similar for both groups (fig. 1). A markedly lower rate of change in the concentrations of protein components was evident by the sixth milking, the retardation seeming to occur earlier in the casein than in the albumin-globulin fraction. A subsequent gradual decline continued to the end of the second week, the time of final sample collection. The changes noted in total protein largely reflected changes in the albumin and globulin.

Non-protein nitrogen also followed a logarithmic decline which, except for the increases from the first to the second milkings, seemed to continue at approxi-

mately the same rate for the first 16 milkings. The physiological significance of the occurrence of higher values at the second than at the first milking is obscure. This increase was observed in colostrum of 14 of the 20 cows, and for each of the remaining animals the decreases from the first to the second milking were less than 0.002 per cent nitrogen.

Previous studies (6, 10), which indicate that colostrum from first-lactation cows is higher in vitamin A than that from cows in later lactations, suggested con-

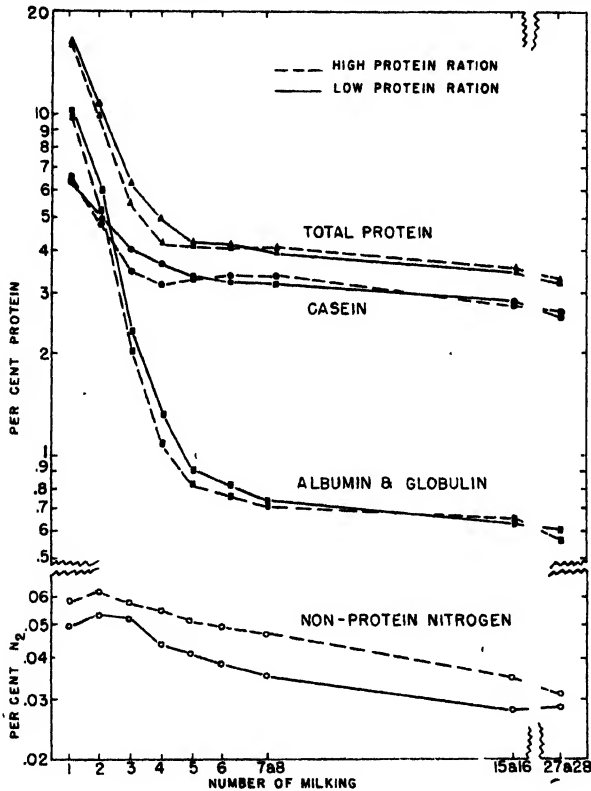


FIG. 1. Distribution of nitrogen fractions of colostrum and early milk from two groups of cows receiving high- and low-protein rations, respectively. Each group is composed of five first- and five second-lactation cows.

sideration of the relation of lactation number to the nitrogenous components of the initial mammary secretions. Analysis of the intra-group data (table 1) indicates that first-lactation cows receiving the high-protein ration secreted more albumin and globulin in the mammary products, particularly in the first three milkings, than did second-lactation cows of the same dietary group. These differences were reflected further in total protein. Concentrations of albumin-globulin nitrogen, however, were similar in the mammary secretions from first- and

TABLE 1
Distribution of nitrogen fractions of colostrum and early milk from groups of cows receiving either a high- or a low-protein ration

Ration	Lactation	No. of milking									
		1	2	3	4	5	6	7 + 8 ^a	15 + 16 ^a	27 + 28 ^a	
Total protein, %											
High protein	1 ^b	19.86 ± 2.98 ^a	13.03 ± 3.47	6.67 ± 1.39	4.52 ± 0.37	4.30 ± 0.41	4.30 ± 0.19	4.18 ± 0.29	3.60 ± 0.25	3.39 ± 0.19	
	2	12.95 ± 1.60	6.81 ± 1.41	4.30 ± 0.82	3.95 ± 0.55	3.89 ± 0.36	3.96 ± 0.35	3.85 ± 0.29	3.42 ± 0.34	3.04 ± 0.40	
Low protein	1	16.74 ± 1.73	10.95 ± 1.70	6.04 ± 1.10	4.46 ± 0.66	3.94 ± 0.32	3.90 ± 0.16	3.72 ± 0.26	3.44 ± 0.18	3.02 ± 0.38	
	2	16.29 ± 5.12	10.39 ± 4.67	6.50 ± 2.09	5.42 ± 1.19	4.50 ± 0.74	4.32 ± 0.50	4.10 ± 0.44	3.47 ± 0.59	3.30 ± 0.16	
Casein, %											
High protein	1	6.81 ± 1.64	5.44 ± 0.97	3.75 ± 0.55	3.23 ± 0.17	3.36 ± 0.26	3.47 ± 0.16	3.43 ± 0.25	2.93 ± 0.16	2.75 ± 0.18	
	2	6.34 ± 0.79	4.15 ± 1.24	3.20 ± 0.59	3.09 ± 0.42	3.17 ± 0.27	3.25 ± 0.30	3.18 ± 0.23	2.80 ± 0.26	2.56 ± 0.33	
Low protein	1	6.32 ± 1.13	5.59 ± 0.79	3.66 ± 0.72	3.23 ± 0.35	3.10 ± 0.16	3.08 ± 0.34	3.05 ± 0.20	2.82 ± 0.09	2.47 ± 0.29	
	2	6.19 ± 1.32	4.39 ± 0.72	4.31 ± 0.93	4.02 ± 0.76	3.54 ± 0.51	3.41 ± 0.44	3.30 ± 0.34	2.85 ± 0.54	2.65 ± 0.13	
Albumin plus globulin %											
High protein	1	13.06 ± 1.88	7.62 ± 2.76	2.90 ± 0.98	1.29 ± 0.31	0.94 ± 0.19	0.83 ± 0.13	0.74 ± 0.07	0.67 ± 0.10	0.62 ± 0.14	
	2	6.73 ± 1.16	2.66 ± 0.19	1.15 ± 0.24	0.86 ± 0.14	0.72 ± 0.11	0.70 ± 0.13	0.67 ± 0.08	0.62 ± 0.08	0.48 ± 0.06	
Low protein	1	10.44 ± 2.63 ^c	5.38 ± 1.33	2.31 ± 0.65	1.26 ± 0.32	0.85 ± 0.17	0.72 ± 0.13	0.67 ± 0.10	0.62 ± 0.10	0.55 ± 0.12	
	2	10.10 ± 4.86	6.52 ± 4.81	2.30 ± 1.36	1.39 ± 0.65	0.97 ± 0.27	0.90 ± 0.19	0.80 ± 0.12	0.62 ± 0.06	0.65 ± 0.13	
Non-protein nitrogen, %											
High protein	1	0.061 ± 0.007	0.066 ± 0.009	0.062 ± 0.008	0.060 ± 0.012	0.054 ± 0.006	0.052 ± 0.008	0.049 ± 0.004	0.036 ± 0.004	0.031 ± 0.003	
	2	0.055 ± 0.008	0.058 ± 0.009	0.053 ± 0.009	0.049 ± 0.006	0.048 ± 0.005	0.046 ± 0.003	0.044 ± 0.006	0.033 ± 0.006	0.031 ± 0.005	
Low protein	1	0.047 ± 0.008	0.050 ± 0.008	0.051 ± 0.009	0.046 ± 0.006	0.042 ± 0.004	0.041 ± 0.002	0.039 ± 0.005	0.029 ± 0.002	0.032 ± 0.003	
	2	0.052 ± 0.015	0.058 ± 0.015	0.050 ± 0.010	0.041 ± 0.009	0.039 ± 0.008	0.035 ± 0.007	0.031 ± 0.006	0.026 ± 0.003	0.024 ± 0.010	

^a Composite samples.

^b Five cows in each group.

^c Standard deviation.

second-lactation cows receiving the low-protein ration. Furthermore, albumin-globulin values for both heifers and cows of the latter group were between those of the two groups receiving the high-protein ration. Only small differences were noted between the casein concentrations of colostrum of first- and of second-lactation cows in either dietary group.

Levels of non-protein nitrogen in colostrum and early milk from first-lactation cows receiving the high-protein ration were higher than those from second-lactation cows. Similar comparisons of colostrum from cows of the two lactation groups receiving the low-protein ration indicated slightly higher non-protein nitrogen values for first-lactation cows only after the first two milkings.

Deviations from the mean of values of the nitrogenous constituents (table 1) are considerably greater during the early colostrual period than later, as the composition of milk approaches normal, further indicating that colostrum is a more variable product than milk.

As might be expected from studies of normal milk (9), data (not shown) suggested that Guernseys and Jerseys produced colostrum of a slightly higher protein content than did Holsteins and Ayrshires. It is recognized, however, that too few animals were used to warrant conclusions relative to breed differences.

Observations of the development and condition of the mammary glands were made in conjunction with the present study. The severity of edema, as determined by palpation and by macroscopic examination, was more pronounced in heifers than in cows and did not seem to be associated primarily with the prepartal rations the animals received (17). Attempts to correlate the total protein and the albumin-globulin contents of early colostrum with the degree of edema were successful only to the extent that the average of each of these protein fractions was higher in colostrum from the ten cows judged to have the more severe edema than from the ten cows with the less severe edema. It also was found that rate of decline of total protein and of albumin-globulin contents in mammary secretions during the transition period was not related to degree of mammary congestion.

DISCUSSION

The results presented herein are in accord with previous reports indicating that the casein and the albumin and globulin contents of colostrum decrease as it changes to normal milk (3, 4, 5, 13, 15). Grimmer (5) pointed out that the proteins of colostrum tend to decrease according to a logarithmic curve during the transition period. In the present study, the decline persisted at the initial rate for only four to six milkings, a somewhat shorter interval than observed for tocopherols (11) and for vitamin A and carotenoids (10).

The effect of high-protein intake on the concentration of non-protein nitrogen of colostrum is similar to that reported for milk (7, 12). The increased protein consumption raised the non-protein nitrogen, not only of the colostrum but also of the blood serum (2). Determinations of the kinds of non-protein nitrogen in mammary secretions and in the blood serum were not made. Other investigators (1) found that in milk from cows fed iodinated casein, urea constituted

approximately one-half of the non-protein nitrogen; whereas in blood plasma, urea frequently accounted for two-thirds or more of the non-protein nitrogen. Results from feeding high- and low-protein rations to cows in normal milk production indicated that a major portion of the increase of non-protein nitrogen of milk from cows fed the former ration was attributable to urea (12).

Differences in the albumin-globulin concentrations in colostrum from first- and second-lactation cows receiving the high-protein ration are too large to be dismissed as a chance occurrence, but interpretation of results is obscured by the fact that similar differences did not occur in colostrum from the two lactation groups receiving the low-protein ration. Although protein quality is not considered to be an important nutritional factor in the case of ruminants, the fact that the proteins in the two diets were not the same might have contributed to differences in the nitrogen fractions of colostrum from cows of the two experimental groups.

Antibodies, which are believed to be important for the well-being of the newborn, are transmitted from cow to calf through the globulins of colostrum (8, 16). Hypoproteinemia is reported to cause a decrease of antibodies and a lowered resistance to infection (16). This observation raises the question of whether first-lactation cows receiving the high-protein ration provided increased amounts of antibodies along with the high levels of albumin-globulin nitrogen of their colostrum.

SUMMARY

Twenty pregnant heifers and cows were paired according to breed and to number and stage of gestation. For 7 weeks (average) before parturition, one group received a high-protein ration and the other a low-protein ration. The rations did not effect a significant difference in the levels of total protein, of casein, and of albumin-globulin fractions of colostrum and early milk from the foregoing groups. Non-protein nitrogen levels were higher in colostrum and early milk from cows of the high-protein group, but the differences were significant only in samples after the first three collections postpartum.

The decrease in concentrations of the protein fractions of mammary secretions during the transition period tended to follow a logarithmic curve for the first four to six milkings, after which the rate of decline was less rapid. Changes in non-protein nitrogen seemed to continue at approximately the same logarithmic rate in samples representing the second through the sixteenth milkings. Rates of change of the nitrogenous constituents were similar in colostrum and in milk from cows receiving either the high- or the low-protein rations.

Analysis of intra-group data indicated that colostrum from first-lactation cows receiving the high-protein ration contained higher levels of albumin-globulin nitrogen than did second-lactation cows receiving the same ration. Only small differences were observed after the first four milkings. Similar differences between heifers and cows fed the low-protein ration were not evident; the values for both of these lactation groups were between those of the heifers and cows receiving the high-protein ration.

Deviations from the mean of values for nitrogen fractions for individual cows within the various groups were considerably greater during the early colostrum period than later, as the composition of milk approached normal.

Total protein and albumin-globulin contents of early colostrum were related to degree of mammary edema only to the extent that the averages of each of these protein fractions were higher from the ten cows judged to have the more severe edema than from the ten cows with the less severe edema. The rate of decline of total protein and of albumin-globulin contents of mammary secretions during the transition period was not related to the degree of mammary edema.

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THE VALUE OF WINTER PASTURE AND SWEET POTATO MEAL FOR LACTATING DAIRY COWS¹

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During recent years, considerable attention has been devoted to the value of winter grazing in the South as a means of reducing feed costs. In many of the southern states, it is estimated that the acreage utilized for winter pasture crops has more than doubled during the past 5 years. This important trend is destined to have a significant influence on the future of the dairy industry in the South.

Therefore, the present study was made in an effort to secure information about the comparative economic and physiologic value of winter grazing versus dry roughage feeding for lactating dairy cows under Georgia conditions. Furthermore, the experiment was so designed that additional information also could be secured on the feeding value of sweet potato meal. A previous study (5) showed no significant difference in the milk and butterfat production or in the liveweights of dairy cows when they were fed a grain mixture consisting of 36 per cent corn or sweet potato meal. Sweet potato meal also was observed to be as palatable as corn when fed in this proportion.

REVIEW OF LITERATURE

Various investigators have pointed out some of the beneficial effects of good grazing upon the quantity (13, 15) and quality (2, 3, 4, 6, 7, 9, 10, 11, 16, 17) of milk produced and the economic aspects (12, 18) of milk production during the seasons of the year when pasture can be provided.

Neel (13) calculated that Balboa rye provided grazing on an average of 169 days per winter in certain sections of Tennessee. A crimson clover and rye grass winter pasture at the University of Georgia Dairy Farm carried at least one cow per acre for 198 days during the winter months of 1946.

Hodgson *et al.* (8) observed an increase in milk production of about 25 per cent when the cows were changed from dry roughage feeding to pasture.

The work of Smith (18) showed that the dairy cow obtained 84.1 per cent of her total feed requirements from grazing during the pasture season of 204 days in the limestone area of southern Indiana. One hundred lb. of digestible nutrients were furnished by silage and hay at a cost of 92 cents and \$1.08, respectively. On the other hand, 100 lb. of digestible nutrients were obtained from pasture at an average cost of 20.1 cents. Calculated on the average cost per 100 lb. of digestible nutrients of feeds other than pasture fed to all livestock

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over a 5-year period, permanent pasture provided \$8.00 worth of feed at a cost of \$1.56 per acre or 19.7 cents per 100 lb. of digestible nutrients.

In studies conducted by the United States Department of Agriculture (17), pasture furnished about 33 per cent of the total nutrients required by cows producing market milk, while constituting only about 14.1 per cent of the total feed cost. Moore at the Mississippi Station (13) reported that the feed cost was \$1.21 per 100 lb. of milk produced when the cows were fed harvested roughages as compared with 69 cents (not including the cost of pasture) when the cows were provided pasture.

EXPERIMENTAL PROCEDURE

Fifteen cows of the University of Georgia Dairy Herd (three Guernseys and 12 Jerseys) were selected and balanced into three groups as evenly as practicable on the basis of age, breed, weight, current production, number of previous lactations, length of dry period and number of days fresh at the start of the experiment. These groups were divided further into five similar outcome groups. The cows were fed identical rations (ration A) during a 14-day preliminary period prior to being placed on the experimental ration (table 1).

TABLE 1
Rations used in study

Ingredients	Ration A	Ration B	Ration C	Ration D
Concentrate				
Ground corn	200	200	200	
Sweet potato meal				200
Oats	200	200	200	200
Wheat bran	100	100	100	100
Soybean meal	150			
Cottonseed meal		150	150	150
Steam bone meal	6	6	6	6
Salt	6	6	6	6
Roughage				
Silage, lb.	6 per cwt.			
Hay, lespedeza	none	ad lib	2 lb	ad lib
Winter pasture ^a (grazing hr. daily)	none	8	none	none

^a Seeding rate was 5 bu. oats, 2 bu. barley and 20 lb. vetch per acre. Fertilizer (10-4-2) was applied at the rate of 200 lb. per acre at time of seeding on Oct. 28, 1946.

Rations B and C were composed of the same constituents except that grazing was provided in addition when the cows were being fed ration B. Rations C and D were of the same composition except that corn was supplied as the main source of carbohydrate in ration C, while sweet potato meal was the main source of carbohydrate in ration D.

The study was conducted during the winter of 1947 for three periods of 28 days each. A 4-day change-over period preceded each experimental period for the purpose of counteracting partially any carry-over effect that the previous ration may have caused and to give the cows an opportunity to become adjusted to the change in feed. The animals were quartered either in the stanchion barn or in a dry lot during the entire time of the study except one group which was

on a 22-acre winter pasture during the day. The pasture group (fed ration *B*) was permitted to graze approximately 8 hours each day, in addition to being fed lespezeza hay and concentrates.

Collection of milk samples. Samples of milk were collected from each cow during the 14-day preliminary period and scored for flavor for the purpose of eliminating any cow that might be giving abnormally-flavored milk. Composite evening and morning milk samples were taken from each feed group twice weekly during the experiment. The samples were placed in storage at a temperature of 40° F. and scored within 15 hours. The butterfat test of each cow's milk was determined biweekly.

Feed samples. A composite sample of the concentrate mixture fed during each experimental period was analyzed for the percentages of moisture, fiber, crude protein, ash, fat and N.F.F.

RESULTS

Physiological condition of the cows. The health and general condition of the cows throughout the study were excellent, except that two cows developed mastitis soon after the study was started. Milk from these cows was not used in the flavor study. Recurrence of this condition throughout the study made it necessary to calculate the milk and butterfat production data of these cows according to the Yates method (20).

The feces of the cows that were fed rations *C* (containing 30.2 per cent corn) and *D* (containing 30.2 per cent sweet potato meal) were firm in contrast to the loose, slightly watery feces of cows that received ration *B* (containing pasture). The liveweight data collected showed no definite trend for or against either of the three rations.

Feed consumption and palatability. All the rations were eaten readily the first time that they were offered. The total roughage consumption of the cows that were fed rations *C* and *D* was 8,003 lb. and 8,154 lb., respectively. Obviously, the small difference between the roughage consumptions of the two groups would be statistically non-significant.

When the cows were allowed to graze winter pasturage an average of 8 hours daily (ration *B*), they consumed only 4,358 lb. of dry roughage or 45.6 per cent (1.82 tons) less hay than did the cows on ration *C*. With hay selling at \$35.00 per ton, the winter grazing had an average value of \$4.25 per cow for a period of 28 days as a dry roughage supplement. This does not take into consideration the increase in milk production (to be discussed later) or the decrease in grain consumption which resulted when the cows went on pasture. The greatest percentage of the dry roughage consumed by the cows on ration *B* was eaten at night when green grazing was not available. Even a greater percentage of the nutrients would have been obtained from grazing had the pasture been seeded earlier.

Chemical analysis of feeds. The concentrate part of each experimental ration was analyzed chemically. These data (table 2) show that ration *B* and *C* (containing corn) had an average of 1.43, 0.83 and 1.05 per cent more moisture,

TABLE 2
Chemical analyses of concentrate mixtures^a

Period	Ration	Moisture	Ash	Protein	Fat	Fiber	N.F.E.
		(%)	(%)	(%)	(%)	(%)	(%)
I	B and C	9.33	4.15	16.69	4.08	8.48	57.27
	D	7.88	5.10	16.25	3.24	8.79	58.74
II	B and C	8.90	4.21	19.06	4.21	9.10	54.52
	D	8.06	5.21	18.13	3.09	10.04	55.47
III	B and C	10.86	4.75	17.69	4.48	8.97	53.25
	D	9.97	4.96	16.56	3.30	9.71	55.50
Av.	B and C	9.97	4.37	17.81	4.26	8.85	55.01
	D	8.54	5.09	16.98	3.21	9.23	56.57

^a Chemical analyses of feeds were made by the department of the state chemist.

crude protein and fat, respectively, than did ration *D* (containing sweet potato meal). On the other hand, ration *D* contained 0.72, 0.38 and 1.56 per cent more ash, fiber and N.F.E., respectively, than did rations *B* and *C*.

Milk and butterfat production. The lactation curves (fig. 1) represent the mean milk yields of outcome groups 2, 4 and 5. The data from outcome groups 1 and 3 were not included, because one of the cows in each of these groups, as pointed out earlier in the report, had recurring attacks of mastitis. A decided increase in milk production occurred in every instance when the cows were changed from dry roughage feeding to pasture. Conversely, when the animals were changed from winter grazing to dry roughage feeding, there was a sharp

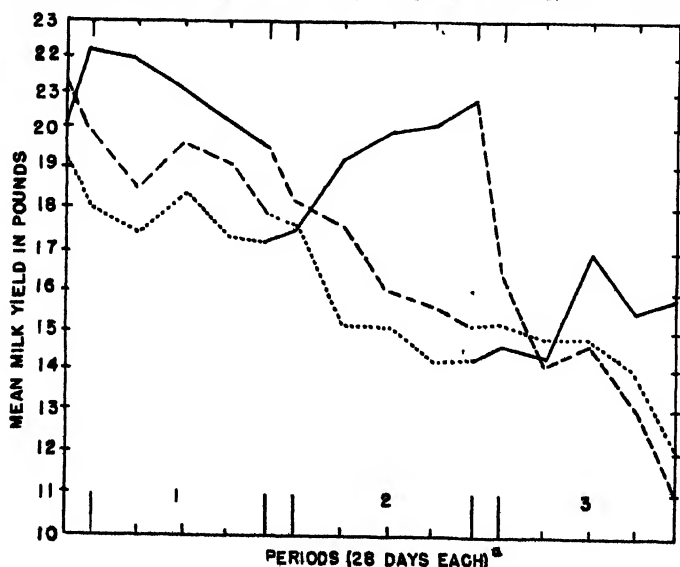


FIG. 1. Lactation curves showing the effect of the test rations on the cows during each of the experimental periods—a 4-day change-over period preceded each experimental period. (Ration B—; Ration C— — — —; Ration D.....)

drop in milk production. The total milk production of the cows³ fed rations *C* and *D* was 8,805 and 8,618 lb., respectively, while the milk yield of those that were fed ration *B* was 10,179 lb. The milk production of the cows while on winter grazing was 15.6 per cent greater than that produced when they were fed ration *C*. The milk yield of the cows fed ration *C* was 2.2 per cent greater than that of the cows fed ration *D*.

The statistical design which made it possible to analyze the milk and butterfat production data statistically was a latin square replicated five times (1, 19). Latin squares, as used in these analyses, refer to outcome groups. Analysis of variance gave an *F*-test (37.08) which indicated a highly significant difference between rations. The mean milk yields per cow when rations *B*, *C* and *D* were fed were 679, 587 and 575 lb., respectively. Application of the *T*-table to determine the least significant deviation revealed a highly significant difference between the population mean milk yield for rations *B* and *C*. Differences in milk yields between rations *C* and *D* were non-significant. The effect of rations on butterfat production was the same as that on milk production. The butterfat yield of the cows while on winter grazing was 18 per cent greater than that produced when they were fed the same type of concentrated mixture and dry roughage. On the basis of the prevailing price (\$1.39 per lb. of grade A butterfat) at the time of the study, the increase in butterfat yield (78 lb.) in favor of ration *B* over ration *C* was worth \$102.86 for the three experimental periods or an average of \$6.86 per cow per period of 28 days. In considering the savings in dry roughage (valued at \$4.25 per cow) and the increased production (valued at \$6.86 per cow), the gross value of the pasture was \$71.75 per cow for a period of 180 days. If the cost of providing the pasture is estimated at \$35.00 per acre, the net returns to a dairy farmer as the result of including winter grazing in his feeding program would be approximately \$36.75 per cow for a period of 180 days. This is a conservative figure because a considerable decrease in consumption of concentrates occurred when the cows went on pasture.

Milk flavor scores and criticisms. The milk produced by cows on winter grazing had a very distinct feed flavor. However, the flavor was very pleasing to the taste except when the cows were milked immediately after being removed from pasture. When the cows were withheld from pasture as much as 12 hours before being milked, the milk was preferred over that produced by cows on dry rations only. No difference was noted in the effect of sweet potato meal and corn on the flavor of milk when the roughage fed was identical.

Composite milk samples were secured from each of the evening and morning milkings four times during each of the experimental periods and scored separately for flavor. The mean flavor scores of the evening composite milk samples when rations *B*, *C* and *D* were fed were 36.2, 37.6 and 36.9, respectively. Samples with no criticism were scored 40 to 45. The *F*-test for ration effect was significant at the 5 per cent level. Application of the *T*-test for least significant differences revealed a significant difference between the mean flavor score of the evening composite milk samples when the cows were fed rations *B* and *C*. The significant

³ Includes missing cow data calculated according to the Yates method (20).

difference in the flavor score of the milk from the cows fed these two rations appeared to be due to a strong grassy flavor which was characteristic of the milk drawn from cows immediately after being removed from pasture. The intensity of this flavor was not as great in the morning milk. The difference in the mean flavor score of evening composite milk samples when the cows were fed rations *C* and *D* was non-significant.

The mean flavor scores of the morning composite milk samples when rations *B*, *C* and *D* were fed were 37.4, 37.0 and 37.5, respectively. Statistical analysis revealed the differences between these data to be non-significant.

SUMMARY

Fifteen dairy cows of the University herd were used to study the value of winter pasture and sweet potato meal for lactating dairy animals during the winter of 1947. The project was conducted for three 28-day periods in accordance with the latin square design (1, 19). Analysis of variance was employed in analyzing the milk, butterfat and flavor data.

The cows that were on winter grazing consumed approximately 46 per cent less dry roughage and produced 15.6 per cent more milk and 18 per cent more butterfat than did the animals that were dry-lot fed. These differences in yields were highly significant.

The mean flavor scores of the afternoon milk samples were 36.2, 37.6 and 36.9 when the cows were fed ration *B* (containing pasture and corn), ration *C* (containing dry roughage and corn) and ration *D* (containing dry roughage and sweet potato meal), respectively. The differences in the flavor scores of the milk from cows fed rations *B* and *C* were statistically significant. The differences in the flavor scores of the milk from cows fed rations *C* and *D* were non-significant. The differences in the mean flavor scores of the morning milk samples from cows fed each of the rations were non-significant.

There was no significant difference in the amount of milk and butterfat produced or in the flavor score of the milk when the cows were fed a concentrate mixture consisting of 30.2 per cent of either corn or sweet potato meal. The animals ate one ration just as readily as the other. The sweet potato meal did not cause an excessive or objectionable laxative effect upon the digestive system of the cows.

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THE DETERMINATION OF BUTTERFAT IN ICE CREAM EMPLOYING MIXED PERCHLORIC AND ACETIC ACIDS

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The Babcock determination of butterfat in milk, cream and certain milk products such as skim milk (2) has been an established procedure for over 50 years. Probably no method of analysis has ever had a record remotely approaching the frequency with which the Babcock test has been applied in the dairy industry.

The unmodified Babcock butterfat test cannot be applied successfully to dairy products containing added sugar due to the charring action of the sulfuric acid. It was the purpose of the present work to show that the use of perchloric and acetic acids in place of sulfuric acid modifies the standard Babcock test to make it applicable to ice cream mix for the determination of butterfat. It can be applied without alteration of existing equipment and with marked improvements in speed, accuracy and simplicity. It diminishes the number of required manipulations per determination, as it is not necessary to add water and the bottle is centrifuged for only a 2-minute period. The increased cost of the mixed perchloric-acetic acid which it employs is more than justified by the saving in time and the abbreviation in operative details. Moderate variation in the amount of acid mixture used does not affect the accuracy of the test.

A mixture of 72 per cent perchloric acid and glacial acetic acid react to form two possible compounds (8), one with the ratio one molecule of perchloric acid to two molecules of acetic acid and the other compound with the molecular ratio of 1 to 1. Such mixtures are not hazardous to mix and may be stored without deterioration. At the boiling point, the acetic acid is evolved and may be thus separated from the perchloric acid. By the process to be described, no precautions other than those applied to the unmodified Babcock test are required. The usual care in the handling of strong mineral acids apply to both procedures.

Sugar is soluble in 72 per cent perchloric acid without charring. Butterfat is as insoluble in aqueous perchloric acid as it is in aqueous sulfuric acid. The proteins of milk and cream are soluble in perchloric acid. Since butterfat in the presence of 72 per cent perchloric acid tends to darken at temperatures near 100° C., thus making reading of the test difficult, it was found desirable to use a mixture of equal parts of 72 per cent perchloric acid and glacial acetic acid as a substitute for concentrated sulfuric acid in the application of the Babcock procedure to the testing of ice cream. The presence of sugar, ice cream stabilizers, flavors and egg products or chocolate does not interfere with the test.

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It is beyond the scope of the present work to give in any detail reference to former procedures which have been developed as substitutes for the original Babcock test as applied to the testing of ice cream. The literature on the subject is very voluminous (1, 2, 4, 5, 6, 7). In no published procedure was found any record of the use of perchloric acid for the purpose of modifying the Babcock test as applied to fat determination in ice cream or ice cream mix.

PROCEDURE

Apparatus and reagents. The perchloric acid-acetic acid mixture, which is the only reagent employed in this modification of the Babcock test, consists of equal parts of volume of 72 per cent perchloric acid ($\text{HClO}_4 \cdot 2\text{H}_2\text{O}$) and 95 per cent glacial acetic acid. Little heat is evolved from the mixing of these chemicals.

Standard Babcock equipment was used to measure the butterfat content by this method. Babcock 20 per cent ice cream test bottles graduated in 0.2 per cent were used throughout this study.

The Mojonnier test, a commercial adaptation of the official Roese-Gottlieb method (3), was employed to carry out control determinations described in this work. All samples tested were evaluated both by the Mojonnier method and the perchloric-acetic acid process simultaneously and the results compared.

The perchloric acid-acetic acid process. The procedure of the test is as follows:

(a) Weigh a 9-g. sample of ice cream mix (or melted ice cream) into a 20 per cent Babcock ice cream test bottle.

(b) Add approximately 30 ml. of the acid reagent (equal parts by volume of 72 per cent perchloric acid and glacial acetic acid) to the test bottle, rinsing the adherent mix off the graduated stem of the test bottle into the body of the bottle as the acid is added. The ingredients should all be at room temperature during mixing.

(c) Digest the ice cream and acid mixture by immersion in boiling water for 5 minutes. No color forms at first, but upon heating in boiling water the mixture turns progressively tan, brown and finally a deep chocolate color. The curd is completely dissolved in 1 to 2 minutes. The mixture should be agitated two or three times during the digestion period. After 5 minutes, the fat will be found as an immiscible supernatant layer.

(d) Add enough of the acid mixture to bring the fat into the calibrated stem of the bottle.

(e) Place the test bottles in balanced pairs in a standard Babcock test centrifuge and revolve at proper speed for 2 minutes. If the centrifuge is heated to 60° C., the per cent of fat can be read as soon as the sample is removed from the centrifuge. If an unheated centrifuge is used, the test bottles should be tempered by immersion in a water bath (130°-140° F.) to the top of the fat column for 5 minutes before reading. The reading of the fat column is made in the customary manner after the addition of glymol.

(f) Contents of the test bottles should be poured into a reservoir of water and then emptied in the sink drain for disposal. The test bottle is rinsed with hot water and is ready for a second test. No coating of insoluble calcium salts ever accumulates on the inner walls of the test bottle. All mineral salts present in cream are soluble in the acid mixture used.

RESULTS

Experimental results on plain vanilla ice cream as compared with the Mojonnier test. Thirty-one different samples of plain vanilla ice cream were subjected to test. These samples were from a wide variety of commercial sources or were

experimental ice creams prepared in the University of Illinois Dairy Technology laboratory. No attempt was made to record their composition. The results are shown in table 1. The maximum deviation between the new method and the

TABLE 1

The analysis of plain ice cream and ice cream mix by the perchloric acid-acetic modified Babcock test and comparison with Mojonnier values

Sample no.	Perchloric acid method		Mojonnier method		Maximum variation from Mojonnier	Average variation from Mojonnier
	No. of analyses	Av. B.F.	Av. B.F.			
		(%)	(%)	(%)	(%)	(%)
1	10	11.33	11.22	+ 0.23	+ 0.11	
2	17	12.37	12.22	+ 0.23	+ 0.15	
3	18	9.05	9.03	+ 0.12	+ 0.02	
4	15	15.15	15.05	+ 0.13	+ 0.10	
5	7	12.03	12.00	+ 0.17	+ 0.03	
6	8	13.41	13.46	- 0.26	- 0.05	
7	15	11.80	11.89	- 0.19	- 0.09	
8	4	12.60	12.61	- 0.11	- 0.01	
9	4	12.15	12.22	- 0.12	- 0.07	
10	6	12.78	12.82	- 0.22	- 0.04	
11	4	11.78	11.69	+ 0.11	+ 0.09	
12	2	13.60	13.61	- 0.01	- 0.01	
13	5	10.56	10.51	+ 0.09	+ 0.05	
14	5	12.20	12.08	+ 0.22	+ 0.12	
15	6	10.13	10.17	+ 0.13	- 0.04	
16	8	11.20	11.08	+ 0.22	+ 0.12	
17	4	10.68	10.65	+ 0.15	+ 0.03	
18	4	12.18	11.89	+ 0.31	+ 0.29	
19	37	12.42	12.49	- 0.19	- 0.07	
20	8	12.42	12.30	+ 0.26	+ 0.12	
21	8	12.48	12.44	+ 0.16	+ 0.04	
22	6	12.37	12.29	+ 0.11	+ 0.08	
23	12	12.51	12.21	+ 0.49	+ 0.30	
24	10	12.62	12.28	+ 0.37	+ 0.34	
25	10	12.70	12.43	+ 0.37	+ 0.27	
26	10	12.77	12.54	+ 0.36	+ 0.23	
27	8	12.91	12.64	+ 0.36	+ 0.27	
28	4	12.33	12.31	- 0.11	+ 0.02	
29	4	11.98	12.02	- 0.12	- 0.04	
30	4	11.63	11.60	+ 0.10	+ 0.03	
31	4	11.13	11.20	- 0.20	- 0.07	
Summary	267					+ 0.07

Mojonnier process was + 0.49 per cent. The average algebraic difference was + 0.07 per cent.

Eight analyses of the same sample gave 11.2 per cent for six determinations, 11.1 for one determination and 11.3 for the remaining test. The Mojonnier test for this sample was 11.08 per cent.

The determination of butterfat in chocolate ice cream. The procedure as described was applied to the determination of butterfat in eight samples of chocolate ice cream with the results given in table 2. Control analyses were carried out using the Mojonnier method. Results of the test of chocolate ice cream samples indicate that the perchloric acid-acetic acid procedure is satis-

factory for use in the determination of butterfat in chocolate ice cream. The average variation between the two methods was -0.11.

SUMMARY

A new reagent has been described for use in a modified Babcock butterfat analysis of plain ice cream and chocolate ice cream. The reagent consists of a mixture of equal parts by volume of 72 per cent perchloric acid and glacial acetic acid. The test requires only one centrifugation and a complete analysis can be accomplished in about 8 minutes. The results are in close agreement with those obtained by the Mojonnier method.

TABLE 2
*The determination of butterfat in chocolate ice cream
by the perchloric-acetic acid procedure*

Sample no.	Perchloric acid method		Mojonnier method		Maximum variation from Mojonnier	Average variation from Mojonnier
	No. of analyses	Av. B.F.	Av. B.F.			
		(%)	(%)		(%)	(%)
1	7	14.36	14.37		-0.17	-0.01
2	12	13.28	13.44		-0.54	-0.16
3	2	20.05	20.14		-0.14	-0.09
4	5	11.00	11.07		-0.17	-0.07
5	4	10.10	10.01		+0.19	+0.09
6	5	15.42	15.12		+0.48	+0.30
7	7	10.89	11.10		-0.30	-0.21
8	7	12.53	13.26		-0.86	-0.73
Summary	49					-0.11

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THE NUTRITION OF THE NEWBORN DAIRY CALF. II. EFFECT OF DIETARY TRYPTOPHAN ON THE URINARY EXCRETION OF NIACIN AND ITS METABOLITES BY YOUNG DAIRY CALVES

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Following the reports (6, 7) that tryptophan can replace niacin in affecting growth of laboratory animals on niacin-deficient rations, various investigators determined the effects of dietary tryptophan on the excretion of niacin and its metabolic products not only in the rat (10, 13) but also in the pig (8), horse (12) and man (11). In all cases the feeding of tryptophan resulted in a marked increase in the excretion of these substances, indicating a metabolic relationship between tryptophan and niacin. The nature and concentration of the excreted products suggest that there is a species variation in this respect. It has been found that there are large increases in the excretion of N¹-methylnicotinamide when tryptophan is fed to rats (13), pigs (8) and humans (11), whereas in the case of the horse (12), there is no significant increase in the excretion of this substance but an increase in free nicotinic acid and other non-methylated products.

In a previous communication (14) from this laboratory, a two-fold increase in the blood tryptophan of calves during the first 3 days of post-natal life was reported. This increase resulted from the ingestion of colostrum which was found to contain an average of 3.85 mg. of tryptophan per g. at the first milking. This is approximately five times that of normal milk on a wet-weight basis. In view of the existing knowledge that a metabolic relationship exists between tryptophan and niacin in the nutrition of the rat, pig, horse and man, and that calves do not require a dietary source of niacin when fed a synthetic milk diet (5), it was considered desirable to study the effects of feeding tryptophan to calves on a milk diet on the excretion of niacin and its derivatives.

EXPERIMENTAL PROCEDURE

Two calves were selected for this experiment and maintained on an exclusive milk diet from birth throughout the experimental period. Calf A, a Holstein, was put on the experiment 24 hours after birth. Calf B, a Guernsey, was assigned to the experiment at 40 days of age. This calf had been kept from the time of birth in a wire-bottomed pen. The usual procedure of feeding colostrum for the first few days was followed in both cases. When the urinary excretion of niacin and its metabolites was found to be fairly constant, each calf was fed 15 g. of L-tryptophan during a 3-day period. The weighed amount of L-tryptophan (2.5 g.) was dissolved in a small amount of dilute sodium carbonate

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solution and carefully mixed with the milk before each feeding. The calves were fed twice daily at 12-hour intervals.

Twenty-four-hour samples of urine were collected after 7.5 g. of L-tryptophan had been fed and again immediately following the last feeding when a total of 15 g. had been fed. Similar urine collections were made 2 and 7 days following the cessation of L-tryptophan feeding.

The amount of tryptophan in the milk consumed was determined each day and the amounts of niacin, other non-methylated products, N¹-methylnicotinamide and tryptophan were determined in each urine sample collected. N¹-methyl-nicotinamide was determined fluorometrically by the method of Huff *et al.* (4). A Coleman photofluorometer was used for the fluorescence measurement. Niacin and its non-methylated products were determined by the chemical procedure of Perlzweig *et al.* (9) with minor modifications. The *p*-dimethylaminobenzaldehyde method of Bates (1) as modified by Graham *et al.* (2) was used for the determination of tryptophan in both milk and urine. The Evelyn Photoelectric Colorimeter with appropriate filters was used for all determinations except N¹-methyl-nicotinamide.

RESULTS AND DISCUSSION

The effects of feeding L-tryptophan on the excretion of niacin and its metabolic derivatives are shown in table 1. It will be noted that following the ingestion of colostrum the excretion of N¹-methylnicotinamide was fairly high (table 1, calf A) but dropped rapidly, whereas the non-methylated products

TABLE 1
The effects of feeding L-tryptophan on the urinary excretion of nicotinic acid and its metabolites in dairy calves

Age	Tryptophan in the milk consumed	Urinary excretion			
		Nicotinic acid	Other non- methylated metabolites	N ¹ -methyl- nicotinamide	Total excretion
(days)	(g./day)	(mg./day)	(mg./day)	(mg./day)	(mg./day)
Calf A, born April 7, 1948					
2	9.86	1.30	2.10	5.18	8.58
5	3.19	1.22	4.06	4.85	10.53
12	3.45	3.51	13.51	1.67	18.09
14 ^a	3.96	3.38	14.17	1.91	19.46
16	4.50	3.84	32.79	2.65	39.28
18	3.62	3.62	48.04	3.35	55.01
21	4.05	4.05	34.01	2.55	40.61
27	4.30	3.10	12.10	2.54	17.74
Calf B, born February 25, 1948					
41	2.03	0.43	5.82	3.12	9.36
48	2.61	0.80	4.30	1.22	6.32
58 ^b	2.16	0.30	1.96	1.54	3.80
60 ^a	1.86	0.42	2.13	1.52	4.07
62	2.58	2.50	13.70	1.92	18.12
63	3.41	3.15	22.95	1.85	27.45
66	3.05	1.27	5.83	1.39	8.49
69	3.20	0.84	3.61	1.70	6.15

^a Following this collection 5 g. of L-tryptophan was fed daily for the next 3 days.

^b Secured.

increased appreciably. The difference between calves in the level of free non-methylated products excreted prior to the tryptophan feeding is not explainable, although it must be remembered that these calves differed in breed and age and in milk consumption. Therefore calf *A* received a larger daily amount of tryptophan.

Following the feeding of 15 g. of L-tryptophan, there was a three- to four-fold increase in the excretion of total nicotinic acid. There was no significant increase in the excretion of free niacin or N¹-methylnicotinamide. The major portion of the increase was in the non-methylated products, and the maximum increase was noted in the collection immediately following the L-tryptophan feeding period. The data indicate that N¹-methylnicotinamide is not the main metabolic product excreted by calves. In this respect, the calf is similar to the horse (3) but different from the rat, pig and man. The Illinois workers (5) found a relatively constant excretion of N¹-methylnicotinamide in calves regardless of whether or not niacin was added to their diet. Following the cessation of L-tryptophan feeding the excretion of total nicotinic acid returned to normal for the individual in 3 to 7 days.

The results of the urinary excretion of tryptophan are presented in table 2.

TABLE 2
The effects of feeding L-tryptophan on the urinary excretion of tryptophan

Calf A			Calf B		
Age	Tryptophan in the milk consumed	Tryptophan excreted in the urine	Age	Tryptophan in the milk consumed	Tryptophan excreted in the urine
(days)	(g./day)	(mg./day)	(days)	(g./day)	(mg./day)
12	3.45	24.2	58	2.16*	67.1
14 ^a	3.90	25.5	60 ^a	1.86	72.8
16	4.5	53.8	62	2.50	118.1
18	3.62	65.2	63	3.40	114.5
21	4.01	52.8	66	3.05	103.2
27	4.30	32.2	69	3.20	82.5

* Following this collection 5 g. of L-tryptophan was fed daily for the next 3 days.

Feeding 5 g. of tryptophan daily for 3 days resulted in an increase in the excretion of tryptophan in urine. This increase could account for only 1 to 1.5 per cent of the intake. It reasonably can be presumed that most of the ingested tryptophan was utilized in the body.

The facts that both colostrum and milk are poor in niacin but relatively rich in tryptophan, and that there is a marked increase in the urinary excretion of niacin and its derivatives following the ingestion of tryptophan suggest that dietary tryptophan serves as a precursor of the niacin required by the calf.

SUMMARY

Five g. of tryptophan were fed daily for 3 days to each of two dairy calves that had been maintained from birth on a whole milk diet. The amount of urinary excretion of nicotinic acid and its derivatives and tryptophan was determined on 24-hour samples and compared with similar data obtained previous to and following the tryptophan feeding.

Tryptophan feeding resulted in a three- to four-fold increase in the excretion of total free and combined nicotinic acid. There was little change in the excretion of free nicotinic acid and N¹-methylnicotinamide. The major portion of the increase was in the non-methylated products. The data indicate that N¹-methylnicotinamide was not the main metabolic product excreted by calves.

The urinary excretion of tryptophan accounted for only 1 to 1.5 per cent of the intake.

Increase in dietary tryptophan results in increased urinary excretion of total nicotinic acid, indicating that tryptophan serves as a precursor of niacin in the young calf as in other mammals thus far studied.

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THE OXIDIZED FLAVOR IN MILK AND DAIRY PRODUCTS: A REVIEW

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The oxidized flavor discussed in this review has been known by a number of descriptive terms. The terms most commonly used to describe the flavor are "cappy," "cardboard," "emery," "metallic," "oily," "oxidized" and "tallowy." The so-called tallowy flavor which develops in fluid milk is not the same as the tallowy flavor which develops in dried milk. The former generally is believed to be caused by an oxidation of the phosphatides, while the latter is caused

by an oxidation of the glycerides. The terms "cappy" and "cardboard" were used because early workers thought that the milk bottle cap was the source of the flavor, while other terms were used in an attempt to describe the taste. The presence of this flavor in milk has caused considerable concern in Europe for a number of years. More recently, it has become of increasing importance in the United States.

The development of the oxidized flavor in milk seems to be the result of a mild chemical oxidation of a minor constituent associated with the fat. A number of authors have presented data which indicate that an oxidation of the phospholipids is responsible for the development of the oxidized flavor. Hereafter, the oxidized flavor will be referred to as *the flavor*.

Golding and Feilman (73) were probably the first to make a study of the flavor in milk. However, Guthrie (88) refers to the work of White who, in 1901, scored butter which had a metallic flavor. The defect observed by White may have been due to active glyceride oxidation, because a metallic flavor usually precedes a tallowy flavor in butter. In this early publication, the author made a thorough study of the development and inhibition of the flavor in milk, skim milk, cream and buttermilk.

THEORIES

The enzyme theory. This theory was proposed by Kende (121) and accepted in whole or in part by the workers in this field for a number of years. He claimed to have isolated an enzyme which he called "oleinase", because it catalyzed the oxidation of the oleic radical of the fat. Sharp *et al.* (192) and Chilson (37) observed that heating milk destroyed an enzyme which oxidized ascorbic acid and at the same time destroyed the enzyme which promoted the development of the flavor. The fact that the flavor increases with decreased storage temperature and the observation that the addition of ascorbic acid (a normal constituent of milk) inhibits development have been used as arguments against this theory. The observation that the flavor may be promoted or inhibited by small changes in pH and E_h also seems to be an argument against the enzyme theory.

The chemical oxidation theory. The conditions under which the flavor develops are those that promote oxidation, such as the presence of air and contamination by metals that are known to be oxidation catalysts. The optimum conditions for development of the flavor are those that cause a mild oxidation, such as low storage temperatures, a limited supply of oxygen, a low concentration of certain oxidizing agents and a limited increase in E_h . It has been observed by many workers that if the intensity of oxidation is too great the flavor will not develop (83, 130, 131). Greenbank (83) postulated and presented data in support of his assumption that the flavor is the result of an intermediate oxidation product, and that the development of the flavor in milk may be inhibited by reducing or oxidizing agents. Recently, Krukowsky and Guthrie (131) have presented data which can be used to confirm the work presented by Greenbank (83). By the careful addition of hydrogen peroxide, these authors were able to promote or inhibit the development of the flavor. Milk so treated to inhibit the

flavor development then was made susceptible by the addition of ascorbic acid. This was repeated a number of times with the same result, but eventually the addition of ascorbic acid had no effect.

The addition of copper, a mild oxidizing agent, promotes development of the flavor, while a low concentration of ferric iron may promote the development of the flavor and a higher concentration inhibit it (83). The addition of reducing agents may inhibit development of the flavor or remove the flavor once it is formed (83). In the latter case, the flavored compound must be reduced before it has become bound to the fat or protein.

THE EFFECT OF THE CHEMICAL AND PHYSICAL PROPERTIES OF THE MILK

The chemical properties of the milk which have been considered as affecting the development of the flavor are E_h , pH, poisoning action and titratable acidity.

Oxidation-reduction potential (E_h). Tracy *et al.* (212) were among the first workers to show a relationship between the development of the flavor and the E_h . They found that the addition of copper caused an increase in E_h of the susceptible samples. These conclusions were verified by Thurston (204), Greenbank (82, 83), and Webb and Hileman (223). The latter were unable to find any relationship between the E_h and the development of the flavor when copper was added to milk from individual samples. The inhibition of the flavor by bacterial growth (15, 202, 203) and by heat has been attributed by Greenbank (83) to the lowering of the E_h . Greenbank's work has been confirmed by Josephson and Doan (119), and Gould and Sommer (78). Larsen *et al.* (133) found no correlation between the E_h and the inhibiting effect of homogenization. The addition of copper and iron to milk may cause a change in the E_h . Copper and ferrous iron are most effective in promoting the flavor (83). Ferric iron and hydrogen peroxide may inhibit if a sufficient concentration is employed (83). The use of poor feed increases the E_h and promotes development of the flavor, while green feed lowers the E_h and development of the flavor is inhibited.

Poising action. Poising is the resistance of milk to a change in E_h ; it is analogous to buffering in the acid-base system. Greenbank (83) concluded that the variation in individual samples is a result of differences in poisoning. When poisoning is used as a criterion, according to Thurston's (205) classification, spontaneous milks are those which are very poorly poised, susceptible milks those poorly poised, and non-susceptible milks those well poised. The difference in poisoning between susceptible and non-susceptible samples seems to be confirmed by the data of Krukovsky and Guthrie (130) on the oxidation of ascorbic acid in susceptible and non-susceptible samples. Greenbank (81) proposed a test to detect susceptibility based on poisoning, which he claimed was 90 per cent accurate. Webb and Hileman (223) were able to predict with a fair degree of accuracy the susceptibility of samples by the rise in E_h after the addition of copper. The reduction of methylene blue in milk by light has been used as an indication of susceptibility (1, 62). Greenbank and Holm (86) have shown that methylene blue dissolved in butterfat is reduced by light.

Hydrogen ion concentration (pH). The effect of pH on the development of the oxidized flavor has not been studied extensively. Greenbank (83) found that an increase in pH of 0.1 was sufficient to inhibit development of the flavor for 24 hours. Although all samples developed the flavor after storage for 24 hours, the samples with increased pH developed less flavor. As a rule, an increase in OH ions accelerates oxidation and may prevent the development of the flavor (83). Anderson (4) presented similar data but attributed inhibition to the activation of an enzyme which destroys the flavor rather than to catalysis of oxidation by OH ions.

Titrateable acidity. Brown and Dustman (22), in a study of 220 samples of milk, were unable to find any correlation between the titrateable acidity and the development of the flavor when the milk was contaminated with copper. Anderson (4) found a relationship between the titrateable acidity and the development of the flavor. Anderson and Triebold (2) observed that reducing milk of high acidity to 0.145 per cent acidity or lower was effective in inhibiting the flavor. Winter milk generally has a higher titrateable acidity than summer milk, which would appear to be a positive correlation with the observation that winter milk is more susceptible than summer milk, but there probably are other changes that are more significant (4).

Color. The variation in the color of milk, especially the yellow color, has been correlated with the development of the flavor. The attempt to correlate color and the development of the flavor is probably a result of the fact that carotene has been thought to be an antioxidant. Anderson (6, 7) and Anderson *et al.* (10) were among the first to find a relationship between color and flavor. The yellow color of milk largely is due to the pigment carotene. Tucker *et al.* (220) found a good correlation between the intense yellow color and good flavor. Whitnah *et al.* (228) found that milk which was below the average color for the breed developed the flavor. However, they also found samples low in color which did not develop the flavor. See "Carotene," and "green feed" for additional discussion.

THE EFFECT OF MILK CONSTITUENTS

Glycerides. The glycerides of the fatty acids are reasonably stable at temperatures most conducive to the development of the flavor. When the glycerides oxidize, there is a measurable decrease in the iodine value. Kende (121) and Dahle (43) found a decrease proportional to the intensity of the flavor. Brown *et al.* (23) were unable to find any decrease. This has been confirmed by Swanson and Sommer (201). Since the flavor is thought to be the result of an oxidation of the phospholipids, no appreciable decrease in the iodine value should be expected, since the concentration of phospholipids in milk fat is low. The same authors found that the oxidized flavor is pronounced in butter, buttermilk, cream and milk, but found only a trace in butteroil. They found that oxidized butteroil when dispersed in skim milk has an off flavor but not an oxidized flavor. Dahle (44) mixed cream or butterfat with skim milk from cows that gave susceptible

milk and the flavor developed, but it developed more rapidly in the sample prepared with cream than in the one prepared with butteroil.

Phospholipids. Whole milk contains from 0.0038 to 0.2889 per cent phospholipids, according to Panzer (159). Early workers pointed out that the flavor developed most rapidly in the cream layer. Guthrie (88), as early as 1916, found that buttermilk from a susceptible cream developed a much stronger flavor than either the cream or the milk. The phospholipid content of milk and milk products (176) decreases in the following order: buttermilk > cream > whole milk > skim milk,—which has also been found to be the order of decreasing intensity of flavor development (88, 207). Thurston *et al.* (208) conclude that the oxidation of lecithin is the cause of the flavor. They arrived at this conclusion because removal of the hulls from milkfat globules, which then were redispersed, resulted in a milk which did not develop the flavor. Roland and Trebler (179) found a decreased sensitivity to copper-induced flavor when mechanical separation was employed. They attributed this to a redistribution of the lecithin between the fat and aqueous phases. Gould *et al.* (77) found no relationship between the lecithin content of the milk and the development of the flavor. Evans (60) found that lecithin (probably impure) was an antioxidant, an observation which has been confirmed by Holmes *et al.* (107) and Koenig (125). Ritter and Nusbaumer (174) found that both lecithin and cephalin of plant origin act as antioxidants. Olcott and Mattill (155) report that lecithin is not an antioxidant, but cephalin acts as such. Much of this work was done on fat substrates and may not be of great value here. Dahle and Palmer (53) conclude that spontaneous flavor, *i.e.* without metallic contamination, is due to the oxidation of the phospholipid fraction of the fat globule membrane. Josephson and Doan (119) found that a typical oxidized flavor develops when phospholipids and copper in suspension are heated together. Phospholipids plus protein developed the flavor without heat, but the intensity was greater when heat and copper were used. Swanson and Somner (201) found a decrease of 30 per cent in the iodine value of the phospholipids from milk which had developed the flavor. The data presented in these studies seem to indicate that oxidation of the phospholipids is the cause of the flavor.

Carotene. Milkfat contains 0.20 to 0.86 mg. of this yellow pigment per 100 g. of fat (176). The reason for the study of the effect of carotene on the development of this flavor is probably its purported antioxygenic activity. Briggs (20), Koenig (126) and Newton (154) conclude that carotene is an antioxidant, while other workers (19, 21, 87, 101, 156) conclude that it has no effect or is a prooxidant. However, most of this work has been done on glyceride substrates and may not be applicable in this work.

Brown *et al.* (30) concluded that some substance associated with the carotene is responsible for the development of the flavor. Trout and Schied (219) found no relationship between the carotenoid content and development of the flavor.

Vitamin A. The concentration of vitamin A in milk varies from 2.5 to 50.0 Sherman units per g. depending on the feed. It is practically all in the butterfat.

Booth *et al.* (18) found the concentration of vitamin A in summer milk was three times as great as in winter milk. Garrett *et al.* (67) found that feeds which increase vitamin A also may increase ascorbic acid. For additional discussion see "Green feed," and "supplements."

Ascorbic acid. In this review, the interest is greater in ascorbic acid than in vitamin C because the latter contains dehydroascorbic acid which does not influence the flavor (131). Milk has been found to contain as high as 26.5 mg./l. of ascorbic acid, according to Riddell *et al.* (168). It is reasonable to assume that ascorbic acid plays some role in the development of the flavor, because it is a reducing agent and has been reported to be an antioxidant. The ratio of the reduced form to the oxidized form may reflect the E_h of the milk because the ascorbic-dehydroascorbic acid system is reversible. Garrett *et al.* (67) found a relationship between the ascorbic acid content and the flavor of milk the day it was drawn. Hand and Sharp (98) found a good correlation between the oxidation of ascorbic acid and development of the flavor. Trout and Gjessing (217) found the ascorbic acid content greater in summer than in winter, which is the opposite of seasonal variation of the flavor intensity. This might indicate that ascorbic acid inhibits development of the flavor.

Whitnah *et al.* (228) found that the relationship between ascorbic acid content and development of the flavor varied in milk from different breeds. This is discussed under "Biological Factors." They found no relationship between vitamin C and development of the flavor in milk from cows within the breed. Sharp *et al.* (192) and Dahle (42) report there is such a relationship. Tucker *et al.* (220) found that a concentration of from 15 to 18 mg./l. was required to impart a good flavor to milk. Brown *et al.* (29) found that feeding KI reduced the ascorbic acid but had no effect on the intensity of the flavor. They made no study of the physical or chemical properties of the milk. Later, the same authors (25) found that the addition of 0.1 g./l. of KI would inhibit. A study of the passage of KI from the feed to the milk would be interesting. Recently, Krukovsky and Guthrie (130) concluded that ascorbic acid is a link in the chain forming the flavor. They based this conclusion on the observation that the oxidation of ascorbic acid by H_2O_2 inhibits development and milk so treated can be made to develop the flavor by adding ascorbic acid. Later, the same authors concluded that it is the "pressures" of ascorbic and dehydroascorbic acid which control the development of the flavor. Greenbank (85) explains these reactions in a slightly different manner. He concludes, according to his intermediate oxidation product theory, that when all the ascorbic acid is destroyed the E_h is high enough to produce a completely oxidized form of the causative agent which has no flavor. The addition of more ascorbic acid to the milk lowers the E_h so that the intermediate or flavored compound may form (83). The correlation of "pressures" of ascorbic-dehydroascorbic acid is another way of expressing the ratio of the reduced to the oxidized form. This ratio is the basis for E_h . These data may be used to confirm Greenbank's (82, 83) conclusion that the development of the flavor is related to the change in E_h . Guthrie *et al.* (92) found a general relationship

between factors which accelerates the rate of oxidation of ascorbic acid and development of the flavor.

Riboflavin (lactochrome) vitamin B₂. The green pigment in whole milk is concerned in a number of ways with biological oxidations, according to Ball (13). When combined with a specific protein, riboflavin becomes an enzyme. Such compounds are called flavoproteins. One of these is Schardinger's enzyme. It is not known whether it plays any part in the development of the flavor, but one interesting fact is that this enzyme is concentrated on the surface of the fat particles. Separating milk concentrates the flavoprotein in the cream and churning the cream concentrates it in the buttermilk (98, 101). It may be significant that flavoprotein is found in the following increasing order: Milk < cream < buttermilk, which is the order of increasing susceptibility to development of the flavor. The acceleration of the photochemical oxidation of ascorbic acid by riboflavin is discussed under "Irradiation."

Proteins, lactose, and salts. These constituents do not seem to be concerned in the development of the flavor, because they are stable towards oxidation under conditions most favorable to the development of the flavor (83).

BIOLOGICAL FACTORS

Biological factors are important because they influence the properties of the milk. In studying the biological factors, it is important that changes in constituents and properties be observed at the same time. Many conclusions found in the literature are not of much value because variables other than the one studied were not controlled.

Breed. The relationship of breed to the development of the flavor has been studied because certain breeds seem to synthesize carotene from their feed more readily than others and thus produce a more highly colored milk. Color has been thought to be related to the development of the flavor. Whitnah *et al.* (228) found that all the samples in which the flavor developed were below the breed average in intensity of color. However, they found samples low in color that did not develop the flavor. They found also that the average vitamin C content of milk from different breeds increased in the following order: Holstein, Ayrshire, Guernsey and Jersey, while the spontaneous development of the flavor decreased in the same order (228). If ascorbic acid acts as a flavor inhibitor, the order of susceptibility given is correct.

Bacterial count. It has been known for some time that milk of low bacterial count is more susceptible than milk of high count, provided all other conditions are the same or similar. It has been postulated that the bacteria utilize the dissolved oxygen and form metabolic products which lower the E_h or are inhibitors. The growth of bacteria is one of the factors which inhibits development of the flavor at high storage temperatures. Thurston and Olson (209) found that milk stored at 38° F. had little bacterial growth and developed the flavor, while a sample of the same milk stored at 52° F. had considerable bacterial growth and did not develop the flavor. Roland *et al.* (178) found that the bacterial counts were generally lower in milk which developed the flavor than in

milk which did not develop it. Greenbank (83) found that bacterial growth inhibits the flavor development and decreases the E_h at the same time. Other workers have obtained similar results (1, 57, 202, 203). A number of workers have concluded that bacterial growth inhibits the development of the flavor (3, 53, 212). However, the evidence indicates that the number of bacteria required to exhaust the oxygen or reduce it to a concentration low enough to inhibit would be sufficient to produce serious bacterial defects (46, 64, 134).

Period of lactation. Brueckner and Guthrie (33) were among the first to study the relation of this factor to the development of the flavor, but they were unable to find any correlation. Rasmussen *et al.* (165) found that the ascorbic acid content of milk is relatively high during the early stages of lactation and decreases to a minimum in about 2 months but rises to a maximum during the latter stages. If ascorbic acid content is the controlling factor (130), milk from the middle of the lactation period should be the most susceptible and late lactation the least susceptible milk. This is contrary to the evidence of Corbett and Tracy (39), who found that milk from the first part of the lactation period is most susceptible, especially in the case of heifers.

Feed. Brueckner and Guthrie (33) were among the first to show that when cows are fed green feed they produce more stable milk than when they are fed dry feeds. This same conclusion has been made by a number of workers (27, 42, 53, 121, 204, 206). However, Hening and Dahlberg (104) found that feeding below the Morrison standard did not affect the flavor. They also found that feeding mangels or beet pulp had no effect on the flavor (103). Majer (137) found fresh "alp" hay in the ration inhibited development. Stebnitz and Sommer (198) found that when cows receive grass as a part of the ration the butterfat becomes more unsaturated and therefore more susceptible to oxidation, and the milk becomes less susceptible to development of the flavor. These data may be used to indicate that the fat is not responsible or that the liquid phase plays some part in controlling development of the flavor. Garrett (66) reports that green grasses or legumes preserved as silage or artificially dehydrated hay are especially desirable in preventing development of the flavor. Bartlett *et al.* (14) found molasses silage of immature grasses or legumes excellent in producing milk highly resistant to the flavor development. Babcock and Haller (12) found that feeding different silages had no effect on the copper tolerances of the milk produced.

Dry feeds. The effect of feeds is dependent to a great extent on the preservation of their nutritive value in drying. Anderson (5) concludes that feeding good machine cured alfalfa changes poor milk to good milk and that poor alfalfa will do the reverse. Brown, *et al.* (30) found that feeding high quality alfalfa with alfalfa meal greatly reduced or eliminated metal induced flavor. Feeding of brown leafy alfalfa did not increase the tendency to develop the flavor. Dahle and Carson (47), on the other hand, found that feeding alfalfa hay produces milk more susceptible to the flavor than milk from cows fed on other roughages. Brown *et al.* (27) found that dry feeding increases the susceptibility and green feeding reduces it. Guthrie and Brueckner (90) found that dry feeds are not

the sole cause of the flavor, because milk from separate quarters of the udder developed different flavor intensities.

Supplements. The feeding of supplements is an attempt to supply essentials that are absent from poor feed. In correlating the feeding of these supplements, it is essential that a study of all the changes in the milk be made, otherwise, the conclusions may be misleading. The feeding of carrots is beneficial in preventing development of the flavor (9). Corbett and Tracy (38) fed cocoanut and corn oil and found that the iodine value of the milkfat was increased markedly, but there was only a slight change in susceptibility. Brown *et al.* (28) fed 1 lb. of cocoanut oil per day. The iodine value of the milkfat increased slightly and there was also a slight increase in susceptibility. One lb. of soybean oil increased the iodine value greatly and increased susceptibility to copper induced flavor. Prewitt and Parfitt (162) fed 14 cows ground soybeans, soybean oil, linseed meal, dried brewers yeast, and none of the milk from these cows developed the flavor spontaneously. However, milk from cows which were fed soybean oil or meal was least susceptible to metal-induced flavor. Brown *et al.* (27) fed 1 quart of lemon or tomato juice per animal per day and reduced susceptibility. The same authors found that feeding 0.5 g. of ascorbic acid per day reduces the tendency to develop the flavor. Brown *et al.* (29) fed 5 g. of KI per day and noted a marked decrease in the ascorbic acid content of the milk but no increase in metal-induced susceptibility. No study was made of changes in the other properties of the milk. Later, Brown and Olsen (25) found that 0.1 per cent KI added to milk would prevent development of the flavor.

Anderson *et al.* (9) found that feeding 8 lb. of carrots per day in the ration was more effective in inhibiting development of the flavor than the addition of 500,000 units of U.S.P. carotene. Whitnah *et al.* (229, 230) and Beck *et al.* (15) found a carotene supplement quickly corrected the tendency for the flavor to develop spontaneously. Brown *et al.* (24) found that a carotene supplement rendered the milk more resistant to the metal-induced flavor. Martin *et al.* (143) fed 1/3 g. of carotene per day for 15 days and increased the color of the milk 60 per cent, with a decrease in the flavor intensity. Brown *et al.* (29) studied the effect of ascorbic acid and carotene as supplements on the development of the flavor but did not study the effect of carotene in the ration on the ascorbic acid content of the milk.

Seasonal variations. Mattick (144) was one of the first to report a seasonal variation in the production of the flavor. He found that the flavor appeared in autumn, winter and spring but never in summer. More recently, the variation in susceptibility between winter and summer milk has been observed by many workers (5, 27, 33, 42, 90, 163, 204, 211, 217, 223). The greater susceptibility in winter, as observed by most workers, seems reasonable as shown by the discussion on green feed and its effect on the flavor. Anderson and coworkers (3, 4) found that the titratable acidity of milk is higher in winter than summer and concluded that there was a correlation between titratable acidity and development of the flavor.

THE EFFECT OF PROCESSING

Processing may change the properties of milk so as to inhibit or promote development of the flavor. In the discussion, an attempt will be made to point out changes in the properties of the milk which may influence the development of the flavor.

Heat treatment. Most workers conclude that pasteurization has little effect on the development of the flavor unless metallic contamination occurs. However, Dahle (43, 42) reported that heating milk to 145° F. for 30 minutes intensified the flavor. Gjessing and Trout (71) concluded that the ascorbic acid was less stable in milk pasteurized by holder methods than in milk pasteurized by using higher temperatures, especially when copper is present. Woessner *et al.* (231) concluded that 20 per cent of the ascorbic acid was destroyed in the holder methods of pasteurization. They also concluded that a temperature of 167° to 185° F. for 15 seconds is required to stabilize ascorbic acid. The heat treatment necessary to inhibit development of the flavor is, according to Kende (121), 185° F. for 5 minutes, Sharp (191), 170° F. for 10 minutes, Dahle and Palmer (53), heating to 170° F. Kende (121) and Sharp (185) concluded that heating kills an enzyme which promotes the oxidation. Greenbank (83) found that heat treatment reduces the E_h and attributes retardation to a reduced E_h . This has been confirmed (78, 119). Gould and Sommer (78) and Gould (75, 76) have shown that sulphhydryl compounds, which are of a reducing nature, are formed when milk is heated to temperatures above pasteurization and these compounds produce the cooked flavor.

Storage temperature. The effect of storage temperature on the development of the flavor is one of the factors which supports the theory that the reaction is a mild chemical oxidation and not enzymatic. The intensity of the flavor increases as the storage temperature decreases. This correlation is paralleled in many chemical oxidations and is contrary to the effect of variations in temperature on enzymatic reactions. Lowering the storage temperature should make the oxidizing conditions milder but has been observed to increase the intensity of the flavor (83). Tracy (210) was probably the first to observe that the flavor developed more rapidly at 4° C. than at 20° C. Greenbank (83) verified these results. Bell (15) observed that concentrated milk held at -17° C. became oxidized more rapidly than at -7° C., and also that the intensity at -7° C. finally decreased while that at -17° C. remained the same. However, Thurston and Olson (209) noticed an oxidized flavor in milk held at 38° F. which showed little bacterial growth. The same sample held at 58° F. showed considerable growth, and the flavor was not detected. Tracy *et al.* (212) found that milk incubated from 1 to 6 hours at 68° F. or at 90° F. and subsequently stored at 40° F. is less likely to develop the flavor than milk stored immediately at 40° F. A number of investigators have worked on the development of the flavor in cream (58, 149, 150, 184, 195). The inhibition at higher storage temperature has been attributed to a number of factors. Kende (121), Tracy *et al.* (212) and Greenbank (83) attribute the inhibition to a lowering of the E_h by bacterial growth. Tracy

et al. (212) also conclude that the bacteria use up the dissolved oxygen, but Sharp *et al.* (190) conclude that to do this there would be a deterioration in flavor due to excessive bacterial growth.

THE EFFECT OF METALLIC CONTAMINATION

Copper. Copper is a normal constituent of milk. However, contamination by this metal has been studied for years (11, 105, 126, 136, 172). Rogers *et al.* (177) probably were the first to conclude that copper contamination caused a more intense tallowy flavor in butter than did iron. Copper is an ideal catalyst for the development of this flavor, because it is a relatively mild oxidizing agent and high concentrations will not inhibit development of the flavor (83). Golding and Feilman (73) were probably the first to report the development of a metallic flavor in milk passed over a detinned cooler. Hunziker and Hosman (114) were among the first to point out that copper contamination produces a more intense flavor in milk than does contamination by iron. The mechanism concerned in the action of copper has been studied by a number of investigators. Osborne and Leavenworth (158) and Vandeveld (221) found that copper combines with protein more as an absorption complex than as a chemical compound. Olson and Brown (157) found that copper combines with the ascorbic acid anion and thereby promotes oxidation; Brown *et al.* (26) found that the intensity of the flavor was greater when copper was added after rather than before pasteurization. This has been confirmed by Greenbank (83).

Iron. This metal, like copper, is a normal constituent of milk. Contamination by iron is not as detrimental as contamination by copper. This has been observed by many workers (26, 83, 136). It has been shown that ferrous iron promotes the development of the flavor but requires a much higher concentration than does copper (83, 201). Ferric iron is less effective than ferrous iron and may even inhibit the flavor (83). Samples containing ferric iron were found to possess the flavor after storage for 24 hours but not for 48 hours (83). Hartman *et al.* (100) found ferrous iron lowered the E_h and did not cause as intense a flavor as copper. This has been confirmed by Swanson and Sommer (201). The effect of copper and iron on the development of the flavor has been studied by a number of workers (36, 41, 54, 55, 70, 72, 74, 80, 88, 93, 128, 142, 147, 148, 150, 171, 194, 195, 222, 225).

Other metals. Besides those already discussed, aluminum, lead, nickel, tin and zinc are the metals used in pure form or as alloys in dairy equipment. Nickel has been studied extensively by a large number of workers (26, 36, 41, 54, 55, 59, 63, 68, 70, 74, 93, 132, 146, 171, 215, 226, 227). Guthrie *et al.* (94) report the development of the flavor by nickel contamination in one case. Whitefield *et al.* (227) report a metallic flavor was caused by contamination by nickel. Fink and Rohrman (63) found that during pasteurization nickel may replace copper that is in solution and render the milk less likely to develop the flavor. Aluminum has been found by many workers to be without effect (63, 68, 94, 109, 112, 129, 171, 225, 227). Allegheny metal, chromium nickel steel and stainless steel are not corroded by milk and do not promote development of the flavor (109, 112, 113, 122, 164, 225, 226, 227).

Manganese, lead and zinc do not influence the oxidation of ascorbic acid and do not affect the flavor.

Irradiation. The exposure of milk and dairy products to light is a form of irradiation. The effect of light on the flavor of dairy products has been studied since 1890 (95). A large number of workers have observed the effect of sunlight on milk (36, 65, 140, 141, 145, 163, 206). The effect seems to be dependent upon the intensity, wave length and time of exposure (83). Hand *et al.* (97) have verified Hopkin's work (108) which indicated that the riboflavin of milk catalyzes the photochemical oxidation of ascorbic acid and itself is changed in the reaction to lumichrome. After the destruction of the riboflavin, the ascorbic acid is stable to the action of light (97, 108, 124). The addition of more riboflavin restores the reactivity (98). Burr (35) probably was the first to observe that exposure to light hastened the deterioration of milk and that dark bottles would prevent deterioration. Hammer and Cordes (96) confirmed Burr's work and added that copper and iron hastened the development of the flavor. Sharp *et al.* (187) found that part of the vitamin C is destroyed by sunlight if oxygen is present, but if the milk is deaerated the vitamin C is not destroyed. They also found that irradiation to produce vitamin D in milk decreases the vitamin C content from 3.4 to 1.7, 3.8 to 1, and 11.0 to 5.5 mg./l. Trout and Gjessing (217) found a slight destruction of vitamin C by irradiation. Guthrie *et al.* (92) found that paper bottles decrease the effect of sunlight on the oxidation of ascorbic acid and on the development of the flavor. On the contrary, Doan and Meyers (56) found that the flavor is more intense in milk stored in paper than in milk stored in glass bottles, but paper bottles did protect against development of burnt flavors. It would appear as if the difference here is one of light transmission or intensity of the incident light and that the bottles used by Doan and Meyers transmitted some of the shorter wave lengths which cause burnt flavor (83). Marquardt (139) found that 20 to 60 minutes in sunlight caused the flavor to develop in 24 hours and 2 hours caused a bleaching effect. According to Greenbank (83), light may inhibit, promote or have no effect on the development of the flavor, depending on the metallic contamination of the milk and the intensity of irradiation.

Dissolved gases. Fresh whole milk drawn without gaseous contamination contains dissolved gases of which 81.5 per cent is CO₂, 2.42 per cent O₂ and 16.54 per cent N₂ (168). Milking increases the O₂ content to 13.18 per cent. Guthrie (89) found that milk direct from the udder contains from 0 to 11 mg./l. of O₂. According to Sharp *et al.* (190), hand milking introduces 5.8 and machine milking 4.7 mg./l. of oxygen.

The effect of air on the deterioration of milk and dairy products has been studied for years (95, 117, 118).

Hartman and Garrett (99) found that the ratio of oxygen consumed to ascorbic acid oxidized increases progressively as the oxidative reaction proceeds. After the ascorbic acid is oxidized, there is a further consumption of O₂, presumably by the oxidation of the fatty substances. Greenbank (83) observed that aeration, and Sharp *et al.* (190) that deaeration inhibited development of

the flavor. These reactions are discussed under the heading "Prevention." Thurston *et al.* (208) and Greenbank (80) found that prolonged stirring inhibits the flavor. This may be assumed to be a form of aeration. Deoxygenation by bacteria is discussed under "bacterial count."

Homogenization. Tracy *et al.* (212) were probably the first workers to observe that homogenization retards the development of the flavor. More recently, Thurston *et al.* (208), Dahle (45) and Ross (180) observed the same effect. Trout and Gould (218) report that homogenization does not retard development when the copper contamination is too high. Larson *et al.* (133) confirmed the previous work but found no relationship between extent of inhibition and the changes in E_h . While there is not a direct correlation at every point between the flavor and E_h , the work does show that the E_h of the homogenized samples maintains a relatively high potential for at least a week, while the unhomogenized samples show a marked drop in potential after the first day (133).

METHODS OF PREVENTION

Aeration and deaeration. The results of a number of workers seem to prove quite conclusively that the development of the flavor may be inhibited by either aeration or deaeration (53, 80, 190). Greenbank (80) was able to inhibit the development of the flavor by aeration or addition of hydrogen peroxide. The same author was able to increase copper tolerance by aeration. Prolonged agitation, which is a form of aeration, has been found by Thurston *et al.* (208) to inhibit development of the flavor. Dahle and Palmer (53) were probably the first to conclude that removal of oxygen dissolved in the milk would prevent development of the flavor. More recently, this conclusion has been confirmed by other workers (91, 92, 97, 188, 189, 190). Sharp *et al.* (187) have developed a commercial method of deaeration which protects the milk for 7 days when contaminated with 0.1 mg./l. of copper. Brown *et al.* (32) found that the flavor developed most rapidly in vacuum capped bottles. Greenbank (83) found that deaeration would not protect against flavor development when relatively high concentrations of copper were present.

Elimination of metallic contamination. Much of the oxidized flavor in milk would be eliminated if metallic contamination did not occur. Roadhouse (175) found that passing 5 gallons of hot milk through a bronze pump caused the development of the flavor and loss of 2 points in score. Nickel in the equipment may replace copper in solution, according to Fink and Rohrman (63). The use of stainless steel and enameled equipment practically eliminates the possibility of the flavor developing from contamination.

Segregation. A simple method of preventing the development of the flavor is to eliminate individual samples of milk which are "spontaneous" or develop the flavor with low metallic contamination. Spontaneous milk may be detected by storing individual samples at 4.0° F. Susceptible milk of low copper tolerance may be detected by the increase in E_h after the addition of copper, according to Greenbank (81).

Antioxidants. Although the addition of antioxidants to milk is prohibited

by law, a number of workers have studied the action of these compounds in milk. Ritter and Christen (173) used a dried culture of bacteria which Kertesz (123, 124) called "*Reductobacterium frigidum neutrale*" and inhibited the development of the flavor. The authors isolated 5 to 7 per cent hydroquinone from the dried culture. Bird *et al.* (17) found that the higher the content of iron the less tendency for the flavor to develop. They believed that the iron combines in a ferrous form with the protein to serve as an antioxidant. Anderson (8) reported the use of pancreatic enzyme prevented development of the flavor whether metal contaminated or not. Russell and Dahle (182) found that concentrated or dried milk added to fluid milk acted as an antioxidant. Dried milk is more effective than concentrated milk. Ritter (169) found hydroquinone, metol and ascorbic acid inhibited development of the flavor. The effect of ascorbic acid and hydroquinone has been confirmed by Chilson (37), Dahle and Palmer (53) and Greenbank (83). Ascorbic acid sometimes is considered an antioxidant. While it does retard development, there remains the question, according to Greenbank (84), whether this action is that of a reducing agent to lower the E_h or to supply protons to regenerate the natural antioxidants in milk. Oat flour, known as Avenex, has been found by many workers to have antioxygenic properties (32, 34, 45, 51, 52, 153, 160).

Condensing and drying. Corbett and Tracy (39) report that concentrating milk to double the solids content prevented the development of the flavor in the condensed and reconstituted milk. The addition of concentrated or dried milk before pasteurization was more beneficial than addition after pasteurization. The addition of 0.2 per cent solids-not-fat had a noticeable effect on the flavor. Dahle and Folkers (49) and Ross (181) found that ice cream containing dry skim milk did not develop the flavor, but the flavor developed when the ice cream contained condensed skim milk. The inhibiting action of these products is probably due to the high heat treatment they received. Krukovsky and Guthrie (130), in a study of ascorbic acid as a key factor in the development of the flavor, concluded that complete oxidation of the ascorbic acid will inhibit development of the flavor. Previously, Greenbank (83) found that the addition of hydrogen peroxide would inhibit the flavor development but did not relate this action to the destruction of ascorbic acid. He concluded that the inhibition was the result of a more complete oxidation of the precursor, presumably to a tasteless form.

THE OXIDIZED FLAVOR IN DAIRY PRODUCTS

Cream. According to many workers, cream is more susceptible to the flavor development than milk. The fact that the phospholipid content of cream is greater than that of milk may be significant. Cream, which was susceptible to the flavor when incubated according to the method of Tracy *et al.* (212), scored 3.5 points higher than a sample of the same cream stored at 40° F. without incubation. Kooper (127) observed that cream held in rusty cans developed a metallic flavor. Another worker (11) confirmed this. A number of investigators have found that it is not so much the exact temperature, provided it is low,

as the metallic contamination in stored cream which induces the flavor development (58, 149, 150, 151, 184, 200).

Condensed milk. The development of the flavor is not as common in condensed milk as in milk or cream, but it has been observed by a number of workers (57, 69, 111, 167). Sommer and Gebhardt (197) report that the flavor of evaporated milk is destroyed in proportion to the copper content; Corbett and Tracy (40) reported that condensing to twice the solids content prevented development of the flavor in the condensed and the reconstituted milk. They attribute inhibition to the liberation of antioxidants derived from the proteins. The high heat treatment should inhibit the development of the flavor, according to other workers (78, 83, 119).

Ice cream. This product is probably less susceptible to the development of the flavor than cream. Strawberry ice cream seems to be very susceptible. However, Dahle *et al.* (47, 49) found pineapple ice cream just as susceptible. Dahle and Folkers (50) and Tracy *et al.* (213, 214) report that increased amounts of berries delayed the onset. They also found that soaking the berries in the mix retarded the development. Heating the berries used to 150°, 175° and 200° F. or autoclaving the berries at 15 lb. pressure for 15 minutes decreased the flavor in the order given but did not eliminate it. Dahle *et al.* (50) were unable to eliminate the flavor by heating berries for 1 hour at 180° F. Mudge and Tucker (151) reported that aeration of the berries for a considerable time resulted in a stale and unclean flavored ice cream. The presence of metallic salts in the berries has been given as a reason for the development of the flavor. Ross (181) reports that iron is a factor, and Iverson (115, 116) reports that it is not a factor in the development of the flavor. The latter postulates that ferrous iron combines with protein and acts as an antioxidant. Mack and Fellers (136) observed that the acidity of the berries induced flavor development. Tracy *et al.* (214) observed that the citric acid content had no effect. The latter authors found that apples, apricots, lemons, oranges, pineapples and peaches promote the flavor when in concentrations of 3 to 5 per cent. The work of Dahle and Josephson (51) indicates that vanilla ice cream will develop the flavor but not as readily as strawberry. This difference may be due to the vanillin which has antioxygenic properties. Schricker (193) found that the development of the flavor occurs with increasing intensity in the following order: chocolate, vanilla and strawberry. Chocolate is probably protected by the tannins in the chocolate which are antioxidants and as previously stated, the vanilla by vanillin. Dahle and Folkers (48) and Ross (181) found that dry milk has a tendency to inhibit but that in most samples containing condensed milk the flavor developed, probably because dry milk gets the higher heat treatment and contains more reducing substances. Ross (181) observed that condensed milk contained more copper and developed the flavor sooner; Dahle and Folkers (50) found that the flavor in milk or skim milk has little effect on the good flavor of the ice cream. Bird *et al.* (17) found more copper in condensed skim milk than in the skim milk powder; the ice cream made with the powder developed a stronger flavor than ice cream in which condensed

milk was used. Dahle and Josephson (51, 52) report that 0.3 per cent of Avenex was not quite sufficient to inhibit completely the development of the flavor in strawberry ice cream. Mueller and Mack (152) found 0.25 per cent sufficient to delay development of the flavor but 0.50 per cent was still more effective. Weckel (224) suggested the use of not more than 0.3 per cent for vanilla and 0.50 per cent for strawberry ice cream. Mack and Tracy (135) and Burke and Newman (34) and also Brown (31) found that 0.5 per cent of Avenex was sufficient to insure a fresh flavor.

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THE KEEPING QUALITY, SOLUBILITY, AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS. II. SOLUBILITY AND DENSITY¹

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In judging the quality of whole milk powder, first consideration should be given to palatability. Of almost equal importance for proper consumer acceptance is the solubility of the powder and the ease with which it may be re-constituted.

REVIEW OF LITERATURE

Solubility. There are many reports which show that the temperature-time relationship of preheating of the milk has a direct relationship to the solubility of the resulting powder. However, it should be borne in mind that solubility is relative and depends upon the solubility test used.

Crossley and Johnson (4) concluded that the solubility of milk powder was least impaired when the preheating temperature did not exceed 159° F. for 20 seconds. For temperatures of between 150 and 163° F. for 20 seconds, followed by a 3 to 5 minute holding period at a slightly lower temperature, the mean solubilities found were nearly a constant value only just below that obtained at 159° F. Even at 167° F. the reduction in solubility was not of commercial significance. Hollender and Tracy (6) compared the solubility indices of powders made from whole milk preheated at 150, 170 and 190° F. for 30 minutes and found that the least soluble powders were those made from the milk preheated at 190° F. However, Crossley (3), by using the short-time preheating method, found little loss in solubility in powder made from milk preheated at 190° F. as compared to that in powder made from milk preheated at 165° F., and the very small loss of solubility appeared to be more than offset by the increased keeping quality with respect to flavor.

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Wright (20), in studying milks of concentrations varying from 20 to 50 per cent total solids and preheated at 194, 203 and 212° F., found that for a given temperature, the higher the concentration of the milk the more readily was the protein rendered insoluble. He reported that when milk powder containing 2.5 per cent moisture was exposed to temperature-time relationships ranging from 212° F. for 10 hours to 282° F. for 40 seconds, 50 per cent of the casein was rendered insoluble. In another experiment, Howat and Wright (7) heated spray-dried whole milk powder for 6 hours at 221 to 230° F. Sixty per cent of the protein was insoluble when the powder was reconstituted at 68° F. Crossley and Johnson (4) found that prolonged exposure of milk powder to hot air currents, even at 194° F. or less (in bag dust collectors), resulted in significantly lowered solubility.

In 1931, Lampitt and Bushill (10) reported that the decrease in solubility of powders of comparable moisture content (less than 6 per cent moisture) was almost negligible when stored at room temperature and very much slower than at 86° F. Lea and Smith (12) concluded that changes in solubility due to storage at ordinary temperatures for a number of years would be exceedingly slight with gas-packed powders at reasonably low moisture contents. Lea *et al.* (11) found that a moisture content of 2.2 per cent had no appreciable effect on solubility.

Hollender and Tracy (6) stated that when powders were stored at temperatures below 68° F., variations in the moisture content between 2.32 and 5.39 per cent had no significant effect on the solubility of the various powders studied. They observed that those samples which discolored during storage became less soluble, that 5 per cent moisture content is about the critical point and upper limit for samples to be stored at room temperature without discoloration, and that at temperatures of 68° F. or above discoloration takes place regardless of the moisture content. They concluded that conditions favorable for brown discoloration and decrease in solubility of milk powders were preheating the milk at 190° F. for 30 minutes, a moisture content of 5 per cent or higher and storing the powder at temperatures of 68° F. or higher.

Wilster *et al.* (19) listed small particle size, small, uniform-sized fat globules and care not to overheat the powder in the drying chamber as favorable factors for good solubility of whole milk powder.

Density and particle size. Webb and Hufnagel (18) found that the density of milk powder could be increased by increasing the degree of preconcentration. Mook (15) described a patented method in which the milk was preheated at 160 to 170° F., condensed to 35 to 40 per cent total solids, and then superheated at not less than 170° F. and preferably at 180 to 210° F. until slight coagulation occurred. The milk powder from this milk was reported to be of greater density and to reconstitute to a fluid with four to eight times the viscosity of reconstituted milk from powders produced by the usual procedures.

Miyawaki (14) found that milk powder made from milk sprayed without preheat treatment had more and larger air cells than the powder made from

preheated milk. Coulter and Jenness (2) reported that the removal of foam from the condensed milk prior to spraying reduced the amount of entrapped air in the powder particles and that, on the other hand, whipping air into the precondensed milk resulted in greater numbers of air cells and air cell clusters in the powder particles. Studying the effect of preheat treatment of milk upon the density of the milk powder, Stamberg and Bailey (16) found that the densest powder was made from milk receiving the most severe heat treatment. The preheat treatments studied were: 150 and 200° F., and 150 and 200° F., followed by superheating. The holding time during preheating was constant for all samples, but it was not definitely recorded.

Hunziker (8) stated, "It has long been observed that an increase in the concentration of the fresh milk is accompanied by an increase in the particle size of the resulting spray powder." According to Hetrick and Tracy (5) and Hollender and Tracy (6), large-size powder particles were obtained by using a low homogenization pressure, low spray pressure and low spray temperature.

EXPERIMENTAL PROCEDURE

The manufacturing and storage procedures used were given in an earlier publication (13).

Solubility test. The solubility indices of the powders were determined by the method of Cone and Ashworth (1) at intervals of 24 hours and 1, 2 and 6 months following manufacture.

It was noted that powder made from the 40 per cent total solids concentrate had a higher solubility index than the powder made from the 20 per cent total solids concentrate. A portion of the original unconcentrated milk for powders no. 108 and 109 was homogenized and subjected to the solubility test. The homogenized concentrated milk from which powders 94 to 109, inclusive, were made also was subjected to the solubility test and the results compared to the solubility index of the fresh powder. In addition, the homogenized concentrates from which powders no. 108 and 109 were made were examined microscopically to determine if any differences in homogenizing efficiency existed due to differences of concentration of the milk. The per cent of fat globules greater than 5 μ in diameter in given fields under the oil immersion objective of the microscope was estimated by Cone and Ashworth (1) by use of a previously calibrated Whipple eyepiece.

Density and particle size. The apparent density was determined on the powders stored at 45° F. for 6 to 7 months by using the following procedure: Light mineral oil (no. 1) manufactured by the Standard Oil Company was employed as the displacing medium. Eight per cent Babcock milk test bottles were found to serve excellently as improvised pycnometers (17). The Babcock test bottles were carefully calibrated at 25° C. with freshly-boiled distilled water at three different levels between the 6.5 and the 8.0 per cent marks on the necks of the bottles. The level of the bottom of the meniscus was estimated to 0.1 of the 0.1 per cent divisions. The density of the oil was determined by use of Hubbard-Carmick specific gravity bottles and by use of the above-mentioned calibrated

Babcock test bottles. In both cases the average value obtained was 0.8407 with a standard deviation of ± 0.00001 . However, the figure 0.841 was used in the density determinations of the powder since the reading of the meniscus of the oil level to 0.1 of a 0.1 per cent division was an estimation to 0.002 ml. From 7 to 11 g. of the powder were introduced into each calibrated Babcock test bottle by use of a 2-inch glass funnel from which the stem had been removed. The apex of the funnel was placed within the flared opening of the test bottle and the powder in the funnel was vibrated into the bottle by use of a "vibro" glass marking tool. Mineral oil then was added so as to fill the bulb of the test bottle about three-fourths full. The powder and oil were mixed thoroughly by vigorous shaking and then were subjected to 20 inches of vacuum for 20 minutes to remove interstitial and adsorbed air. The above conditions for the removal of interstitial and adsorbed air were found to give constant results, whereas a lesser period of time resulted in the appearance of free air bubbles and a period of 30 minutes did not change the results obtained. Upon removal from the vacuum, the oil level was raised with additional oil to approximately the 7 per cent mark. The samples then were tempered in a $25 \pm 0.1^\circ$ C. water bath. At the end of 30 minutes the levels of the bottom of the menisci were recorded, the bottles were wiped dry and weighed, and the densities calculated.

The solubility effect of the mineral oil on the constituents of the milk powder was checked by determining the specific gravity of mineral oil filtered from completed milk powder density determinations. The oil had been in contact with the whole milk powder 2.25 hours at the time filtration was begun; filtration required an additional 2.75 hours. Triplicate determinations on the specific gravity of the oil gave 0.8409, 0.8409 and 0.8410 as the specific gravity.

Centrifugation also was tried as a means of removing the interstitial and adsorbed air. Although the results obtained were approximately the same as when vacuumization was used, the appearance of very fine particles in the oil meniscus precluded accurate reading of the oil level in the stem of the test bottles.

The sizes of the dry milk particles were determined by examining under the oil immersion objective a mineral oil suspension of the powder placed between a glass cover slip and a glass slide. The particle sizes were estimated by use of a previously calibrated Whipple eyepiece with a grid division of 2.5μ . The number of particles measured per batch varied from 1000 to 5900 particles.

RESULTS AND DISCUSSIONS

Solubility. All solubility indices given in table 1 are the averages of three determinations. The degree of preconcentration has a very distinct effect on the solubility of milk powder. In every comparison of paired milk powders, the powder made from the 40 per cent total solids concentrate had a higher initial solubility and maintained a higher solubility during storage than did the powder made from 20 per cent total solids concentrate. Powders made from either 20 or 40 per cent total solids concentrate showed little if any decrease in solubility when stored at 45° F. for 6 months. However, when stored at 100° F. for 6

months, powders made from 20 per cent total solids concentrates showed a distinct lowering of solubility indices. This decrease was least for the powders made from milk preheated at 160° F. for 30 minutes.

The powders made from the 40 per cent total solids concentrate, except those made from milk preheated at 180° F. for 10 minutes, retained to a remarkable degree their initial solubility when stored at 100° F. for 6 months. The powder made from milk preheated at 180° F. for 10 minutes decreased rapidly in solubility. It is suggested that the denaturing of the casein initiated by the high preheat treatment lowers the solubility of milk powders during storage as is indicated also in the data presented by Hollender and Tracy (6). They found that powders made from milk preheated at 150 and 170° F. for 30 minutes did not de-

TABLE 1
Solubility indices of whole milk powders

Preheat treatment	Milk conc., average	No. of samples	Powder, ave. % moisture	Average solubility index after storage for			
				1 day	1 mo.	2 mo.	6 mo.
(% T.S.)							
Storage at 45° F.							
160° F.	20.2	6	2.7	97.4	97.3	97.4	97.3
30 min.	38.1	6	2.2	98.8	98.5	98.5	98.5
170° F.	21.9	4	2.2	98.1	98.5	98.4	98.3
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.3
170° F.	21.0	6	2.3	97.8	98.5	98.4	98.3
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.4
180° F.	22.8	6	2.3	98.4	98.9	99.0	99.0
10 min.	40.3	6	2.0	99.1	99.4	99.4	99.3
Storage at 100° F.							
160° F.	20.2	6	2.7	97.4	97.2	97.3	96.9
30 min.	38.1	6	2.2	98.8	98.7	98.8	98.5
170° F.	21.9	4	2.2	98.1	97.5	97.6	96.7
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.1
170° F.	21.0	6	2.3	97.8	98.1	98.1	96.9
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.1
180° F.	22.8	6	2.3	98.4	98.1	98.2	96.9
10 min.	40.3	6	2.0	99.1	99.2	99.1	97.4

crease in solubility as did powders made from milk preheated at 190° F. for 30 minutes when stored at room temperature for 67 days.

The preheat treatment of the milk, as used in this experiment, appears to affect the initial solubility of the milk powder only to a very slight degree. The initial solubility of the milk powder made from milk preheated at 160° F. for 30 minutes was slightly lower than that of milk powder preheated at the other temperatures. The initial solubilities of the powders made from milk preheated at 170 and 180° F. for 10 minutes and at 170° F. for 30 minutes were about equal when the powders were made from the 40 per cent concentrate. These results are not exactly comparable to those reported by others (6), who showed that the powder made from milk preheated at 190° F. for 30 minutes was less soluble than the powder made from milk preheated at 150 and 170° F.

TABLE 2

Comparison of the solubility index of homogenized whole milk, homogenized concentrated milk and the powder made from the concentrated milk

Powder no.	Milk and conc. milk (% T.S.)	% of fat globules over 5 μ in diam.	Solubility index	
			Milk	Powder
		10	97.8	
108	12.8 ^a	5	98.9	97.6
109	22.6	less than 1	99.3	97.9
	38.6			

^a Original milk from which concentrates for powders no. 108 and 109 were made.

for 30 minutes. These differences probably are due to the shorter period of exposure and the lower maximum temperature employed and possibly due to the differences in method employed to determine solubility.

Efficiency of homogenization. It appeared from microscopic examination that the more highly concentrated milk is homogenized more efficiently. The per cent of fat globules 5 μ or over in size progressively decreased as the pre-concentration increased. The solubility test (1) when applied to these milks indicated a direct relationship between high solubility indices and efficient homogenization. Under the conditions of the solubility test employed, it is impossible to have a solubility index of 100 per cent. Thus, the unconcentrated, unhomogenized sample shows the lowest solubility index because at least a part of the cream layer is retained as insoluble material (table 2).

In table 3 is shown a further comparison of the solubility indices of concen-

TABLE 3

Comparison of the solubility of homogenized concentrated milk and the powder made from it

Powder no. ^a	Preheat treatment	Milk conc. ^b	Initial solubility index	
			Milk conc. ^b	Powder
		(% T.S.)		
106	160° F.	23.3	98.6	97.9
107	30 min.	40.1	99.5	99.3
98	170° F.	22.8	98.9	98.7
99		38.9	99.4	98.9
100		23.4	98.3	97.5
101	10 min.	42.0	99.1	99.2
104		22.7	99.1	98.5
105		39.4	99.3	99.4
94	180° F.	20.6	98.4	99.2
95		41.6	99.3	99.1
96		21.4	99.1	98.1
97		39.9	99.2	99.1
102		39.0	99.1	99.4
103	10 min.	26.5	99.2	99.3
108		22.6	98.9	97.6
109		38.6	99.3	97.9

^a The powders are paired. The even-numbered powders and the following odd-numbered powders are made from the same milk.

^b Homogenized.

trated milks and the corresponding powders made from them. The solubility indices obtained with the concentrated fluid milk samples no. 94 to 109, inclusive, range from 98.3 to 99.5. Generally, the less concentrated samples gave lower values than did the more concentrated samples. The effect of concentration of the milk on the solubility of the powder was even greater than when the test was applied only to the concentrated milk. More efficient homogenization of the more viscous, heavier concentrate may have resulted in the differences of the solubility indices of the milks and the greater differences observed in the powders made from the 20 and 40 per cent total solids concentrate.

Color and solubility. The powders made in this experiment had moisture contents which ranged from 1.5 to 3.1 per cent. None of the powders was discolored noticeably after storage for 180 days at 100° F. Krienke and Tracy (9) showed that brown discoloration of powder takes place at all storage temperatures, the discoloration increasing with increased moisture in the powder. Discoloration was accompanied by decreasing solubility. Hollender and Tracy (6) stated that it was evident that there was a critical point in the neighborhood of 5 per cent moisture content which represented the upper limit of moisture for samples stored at room temperature without deterioration in flavor and color. However, they found discoloration of milk powders would occur at 68° F. or higher, regardless of moisture content. They stored samples of milk powder with moisture contents varying from 2.32 to 5.39 per cent at 98.6° F. All of their samples discolored within 67 days. The lower average moisture content of the present samples may have prevented noticeable discoloration.

Density and particle size. The apparent density of the powdered milk is important for it affects packing volume and ease of handling as well as ease of reconstitution. The effects on packing volume and ease of handling or packaging are well known in the commercial field.

Webb and Hufnagel (18) found that increasing preconcenration of the milk resulted in powders with increased densities. In agreement with their findings, it was found in this work that with a given preheat treatment there was a direct correlation between density and preconcentration of the milk (table 4). This direct correlation of density with degree of precondensing was noted in every instance except for powder no. 94. In the production of this powder, trouble was experienced with continuous clogging of the air outlet of the spray nozzle. It is believed that this prevented normal atomization of the milk, resulting in a heavier, coarser powder.

Increasing the degree of preconcentration from 20 to 40 per cent total solids resulted in whole milk powder not only of greater density but with a higher percentage of the larger-size powder particles (table 5). Wilster *et al.* (19) stated that one of the factors favorable to reconstitutability of dry milk was small particle size. The powders with the highest solubility indices, as reported in this paper, had the highest percentage of the large size particles. However, it must be noted that in nearly all of the powders examined, 95 per cent of the particles were less than 10 μ in diameter (table 5). All of the

TABLE 4
 Whole milk powder densities

Powder no.	Preheat treat.	Freconc.	Powder		Densities at 25° C.	Av. density	
			% fat ^a	% T. S.		sample	group
		(% T.S.)					
70		21.9			1.161, 1.162, 1.162	1.162	
72		18.0			1.180, 1.178, 1.181	1.180	
76	160° F.	19.9	26.3	97.7	1.176, 1.175, 1.170	1.174	
106		23.3	28.4	97.5	1.152, 1.153	1.153	1.167
	for						
71		38.9			1.214, spilled	1.214	
73	30 min.	38.6			1.216, 1.213, 1.211, 1.212, 1.215	1.213	
77		39.3	26.3	97.8	1.206, 1.209, 1.208	1.208	
107		40.1	28.4	97.6	1.189, 1.188	1.188	1.206
88		19.6	26.5	97.9	1.162, 1.165, 1.163	1.163	
92		20.5	26.3	97.9	1.163, 1.160, 1.162	1.162	
100	170° F.	23.4	29.4	97.6	1.135, 1.136	1.136	
110		23.9	28.3	97.6	1.155, 1.158, 1.157	1.157	1.154
	for						
89		42.2	26.6	98.2	1.198, 1.197, 1.201	1.199	
93	10 min.	41.3	26.5	98.5	1.198, 1.199	1.199	
101		42.0	29.5	97.9	1.185, 1.188, 1.186	1.186	
111		37.4	28.3	97.5	1.193, 1.193, 1.192	1.193	1.194
74		20.8			1.178, 1.178, 1.177	1.178	
78		22.0	26.4	97.6	1.169, 1.170, 1.170	1.170	
80		21.0	26.1	97.7	1.164, 1.165, 1.165	1.165	
82	170° F.	19.3	26.1	97.8	1.159, 1.160	1.160	
84		20.3	26.1	97.9	1.158, 1.158	1.158	
104		22.7	27.9	97.5	1.157, 1.155	1.156	1.165
	for						
75		44.4			1.195, 1.192, 1.193	1.193	
79	30 min.	40.7	26.5	97.9	1.204, spilled	1.204	
81		40.3	26.2	98.2	1.206, 1.209	1.208	
83		40.3	26.2	98.1	1.199, 1.195	1.197	
85		40.3	26.1	98.0	1.202, 1.201, 1.202	1.202	
105		39.4	27.9	97.7	1.192, 1.194	1.193	1.198
86		22.0	28.1	97.8	1.164, 1.159	1.162	
90		22.1	26.7	97.4	1.154, 1.157	1.156	
90 ^b		22.1	26.7	97.4	1.152, 1.155	1.154	
94 ^c		20.6	28.4	98.1	1.196, 1.194, 1.197, 1.193, 1.195, 1.196	1.195	
98		22.8	28.3	97.9	1.139, 1.140, 1.138, 1.138, 1.141, 1.141	1.140	
103	180° F.	26.5	28.3	97.2	1.162, 1.164	1.163	
108		22.5	29.0	97.6	1.151, 1.151	1.151	1.154
	for						
87		41.6	28.2	98.4	1.189, 1.190	1.190	
91	10 min.	41.9	27.8	98.2	1.196, 1.197	1.197	
95		41.6	27.5	98.0	1.182, 1.182, 1.179, 1.182	1.181	
99		35.9	28.3	98.0	1.187, 1.189	1.188	
102		39.0	28.4	97.4	1.189, 1.189	1.189	
109		38.6	29.0	97.8	1.189, 1.188	1.188	1.189

^a Calculated per cent fat based on per cent fat in the fresh milk.

^b Adsorbed and interstitial air removed by centrifugation.

^c During the production of this powder, the air outlet of the atomizing nozzle was apparently out of adjustment and became clogged continuously, preventing normal atomization. This result, therefore, is not included in the average.

powder produced for this experiment may be classified as of small particle size, for Hunziker (8) indicates the size of milk powder particles produced commercially by the spray process ranges from 10 to 100 μ , with 80 per cent of the particles being in the relatively coarse group.

In the solubility tests, it was noted that the powders from the 20 per cent concentrate always were highly charged. Even the slightest handling of this lighter powder markedly increased the static charge and made the handling of this powder difficult. Consequently, the dense powder may play a favorable role in the reconstitutability of whole milk powder, because its lesser tendency

TABLE 5
Size distribution of powder particles

Powder no.	Preheat treat.	Preconc.	% of particles—Diameters in microns						
			0-5	5-10	10-15	15-20	20-25	25-30	30-45
(% T.S.)									
72	160° F. 30 min.	18.0	83.0	15.2	1.7	0.1			
70		21.9	79.1	16.3	3.6	0.7	0.24		
73		38.7	68.4	26.2	4.1	0.8	0.53		
77		39.3	61.4	29.7	6.0	1.8	0.50	0.50	
88	170° F. 10 min.	19.6	88.0	11.5	0.5				
110		23.9	82.6	16.5	0.5	0.1			
111		37.4	61.9	31.3	5.5	1.2	0.10		
101		42.0	67.1	25.4	4.5	2.1	0.33	0.47	0.21
74	170° F. 30 min.	20.8	81.7	16.5	1.5	0.4	0.03		
80		21.0	80.6	18.1	1.1	0.2	0.09		
85		44.3	70.4	23.4	4.6	1.1	0.39	0.16	0.02
75		44.4	65.9	27.2	5.1	1.0	0.47	0.16	0.10
94	180° F. 10 min.	20.6	74.9	21.6	3.1	0.3	0.04		
98		22.8	83.4	14.9	1.5	0.2	0.08		
109		38.6	73.9	22.5	2.9	0.5	0.13		
95		41.6	72.2	21.5	3.7	0.9	0.75	0.25	0.57

to build up a high electrostatic charge appears to reduce the wettability of the powder, and the dense powder naturally has less occluded air.

SUMMARY AND CONCLUSIONS

(1) Precondensing milk to a level of 40 per cent total solids resulted in a spray-dried powder of higher solubility than when the milk was precondensed to a level of 20 per cent total solids.

(2) The powders made from the milk of all preheat treatment levels and concentrated to the 40 per cent level reconstituted quickly and without a visible film of specks on the glassware. For all practical purposes, they were 100 per cent soluble at the end of 6 months of storage at 45° F.

(3) The solubility of the powders made from milk preheated at the lower temperatures and precondensed to 40 per cent total solids did not decrease appreciably when stored for 6 months at 100° F. A preheat treatment at 180° F. for 10 minutes appears to induce heat denaturation of the protein, which is con-

tinued when the powder is stored at 100° F., resulting in continued loss of solubility during storage.

(4) A method for quickly and accurately determining the density of whole milk powder is presented.

(5) The density of milk powder increases with increasing preconcentration of the milk. The powders made from milk concentrated to 40 per cent total solids were easier to reconstitute, did not take up as much space, and did not as readily develop a high electrostatic charge as did the powders made from the 20 per cent total solids concentrate.

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EFFECT OF THYROXINE ON OXYGEN CONSUMPTION OF BOVINE SPERMATOOZA AND SEMEN¹

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Previous studies have shown that the rate of oxygen consumption of spermatozoa may be related to fertility (3, 9). Other studies have shown that thyroxine increases the oxygen consumption of some tissues *in vitro* (1, 2). Since certain oxidative mechanisms may play a role in spermatozoan physiology similar to that in other respiring cells (4, 5), the purpose of this experiment was to determine the effect of thyroxine on oxygen consumption and, ultimately, to study the effect of thyroxine on semen fertility. The results obtained with a measured amount of thyroxine on the oxygen consumption of bovine spermatozoa and semen are reported in this paper.

METHODS

Bovine semen was collected by use of the artificial vagina under conditions as aseptic as possible. Experiments with washed and unwashed spermatozoa were carried out. In experiments with washed spermatozoa, the semen was cooled slowly to 4.5° C. immediately after collection and diluted with a phosphate buffer.² The diluted semen was centrifuged and the diluted seminal fluid removed. Phosphate buffer then was added in amounts to make up the original diluted volume. Experiments on unwashed semen were made on samples collected as described above and diluted with a diluent composed of one part fresh egg yolk to ten parts phosphate buffer. The rate of semen dilution varied for different samples. Since preliminary experiments indicated that thyroxine exerts its effect on respiration rate only after it has been in contact with the spermatozoa for several hours, the determinations in these experiments were made 10 to 30 hours after addition of thyroxine to the semen or spermatozoa. Only a limited number of the centrifuged and washed semen samples maintained respiratory activity at a level high enough for a reliable determination after 10 to 30 hours of storage. Preliminary work (7) indicated that from 0.3 to 1.0 γ of DL-thyroxine in 10 ml. of diluted semen brought about an average increase in oxygen consumption. Therefore, 0.7 γ of DL-thyroxine per 10 ml. diluted semen or washed spermatozoa was used in all of the determinations.

The DL-thyroxine was prepared by isolation from iodocasein which was thyroïdally active.³ A weighed amount of this thyroxine was added to distilled water, completely dissolved by adding 0.1 N NaOH drop by drop and this solu-

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² Made up of 20 g. $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$ plus 2 g. KH_2PO_4 in 1,000 ml. boiled distilled water.

³ Supplied by Dr. E. P. Reineke of Michigan State College, East Lansing

tion made up to a volume giving 100 γ thyroxine per ml. For use in treating semen, this thyroxine solution was diluted further with a phosphate buffer so that 1 ml. of solution contained 5 γ . A portion of each semen sample was treated with thyroxine and another portion used as a control. The pH was adjusted so that it was the same in treated and control portions.

Oxygen consumption was determined with a Barcroft-Warburg respirometer at 37° C. The manometers were shaken at a rate of 110 oscillations per minute. Care was taken to mix the semen thoroughly before dividing it into portions. To determine the variability of results due to sampling and operation of the respirometer, the oxygen consumption of two untreated portions of a semen sample was determined. The standard deviation of the difference between two like samples for ten determinations was found to be 1.24 mm.³ oxygen. The concentration of spermatozoa in the original semen was determined with a hemocytometer.

To determine the influence of bacterial contamination on results, oxygen consumption determinations were made on thyroxine-treated and untreated portions of semen in which the spermatozoa had been killed by adding a measured quantity of water to the raw semen before dilution or by warming and cooling of the semen. Also, oxygen consumption determinations were made on treated and control semen to which 800 units of penicillin per ml. had been added. Dilution was one part semen to four parts buffer.

EXPERIMENTAL

The effect of thyroxine in a concentration of 7 γ per cent on the oxygen consumption of bovine spermatozoa and semen is shown for individual semen samples in table 1. On a basis of the oxygen consumed per 100 million spermatozoa per hour, the results show that the average was 8.48 mm.³ for control semen and 9.20 mm.³ for thyroxine-treated semen. These values were obtained with semen stored from 10 to 30 hours at 4.5° C. and with varying concentration of spermatozoa. On the same basis, control semen consumed an average of 8.12 mm.³ oxygen when the concentration of spermatozoa in the original semen was below 800,000 per mm.³, whereas these same semen samples when treated with thyroxine consumed 8.00 mm.³. Apparently, no average change in the amount of oxygen consumed was brought about with the addition of thyroxine. Control semen with a spermatozoan concentration of more than 800,000 per mm.³ and less than 1,400,000 per mm.³ in the original semen consumed 8.66 mm.³ oxygen, whereas these same semen samples treated with thyroxine consumed 9.64 mm.³ oxygen, an average increase of 11.2 per cent.

The results obtained were correlated with spermatozoan concentration of the original semen. In general, as the spermatozoan concentration of the original semen increased from 800,000 to 1,400,000 per mm.³, there was a progressively greater increase in oxygen consumption resulting from the presence of DL-thyroxine. With semen having a spermatozoan concentration of less than 800,000 per mm.³, there was little or no change in the oxygen consumption due to the presence of thyroxine. The correlation coefficient representing the degree of this relationship was found to be +0.60. This is a statistically highly sig-

nificant correlation for the number of determinations involved. This relationship apparently was not due to spermatozoa number *per se*, because the actual number of spermatozoa present in each determination was not related to increasing oxygen consumption resulting from thyroxine treatment. The correlation coefficient representing the degree of the latter relationship was +0.19. Therefore, it is believed that differences in changes of oxygen consumption due to thyroxine are due to variations of some character of semen that is associated

TABLE 1
Effect of 7 γ DL-thyroxine per 100 ml. of diluted semen on oxygen consumption

Sperm concentration in original semen	No. of sperm per 2 ml. diluted sample	Oxygen consumption		% of control
		Control	Treated	
(Thousands/mm. ³)	(Millions)	(mm. ³)	(mm. ³)	
<i>Washed, diluted semen</i>				
700	280	26.56	23.54	88.6
800	266	12.16	14.06	115.6
800	266	24.60	27.68	112.5
900	450	33.47	35.76	106.8
1,000	600	32.06	36.09	112.6
1,160	1,160	29.41	31.22	106.1
<i>Unwashed, diluted semen</i>				
300	120	11.44	11.08	96.9
350	170	14.36	13.84	96.4
450	360	23.34	26.06	111.7
550	220	23.99	23.57	98.3
688	688	36.74	33.94	92.4
700	350	24.28	22.73	93.6
725	482	37.61	42.39	112.7
800	400	75.81	72.63	95.8
820	410	28.58	28.39	99.3
900	360	16.68	21.37	128.0
980	290	21.36	22.86	107.0
1,000	1,000	52.84	59.79	113.2
1,000	100	20.27	22.00	108.5
1,100	220	27.08	32.48	119.9
1,100	440	46.46	48.30	103.9
1,000	440	27.69	34.47	124.5
1,130	900	65.68	75.62	115.1
1,200	480	45.61	50.45	110.6
1,220	490	29.53	34.27	116.1
1,220	1,220	43.25	51.11	118.2
1,375	680	111.60	136.40	122.2

with spermatozoan concentration of the original sample and not to sperm numbers *per se*.

Although not enough evidence has been obtained on semen from individual bulls to draw definite conclusions, it appears that semen from some bulls usually increases in its ability to consume oxygen when thyroxine is present, and semen from these bulls usually has a high spermatozoan concentration. Other bulls seem to produce semen that is depressed or not influenced in its ability to consume oxygen when thyroxine is added and usually is low in spermatozoan concentration. In cells such as bovine spermatozoa that vary considerably in their

physiology, as is indicated by their varying ability to effect fertilization, it is not surprising that the reaction to experimental conditions varies. Varying effects on the oxygen consumption of different tissues due to the influence of thyroxine have been reported by other workers (2, 8).

Bacterial contamination cannot be avoided entirely in semen collection, and its influence on total oxygen consumption may affect results. Therefore, the oxygen consumption of semen samples with the spermatozoa killed was determined for 12 samples. One portion of the sample containing dead spermatozoa was treated with thyroxine and another untreated portion served as a control. The average oxygen consumption of these 12 samples with dead spermatozoa was 3.26 mm.³ per hour, with a standard deviation from the mean of 1.97 mm.³. Actual variation was from an amount of oxygen which could not be detected to 6 mm.³ per hour. For these same semen samples with thyroxine added, the average oxygen consumption was 3.68 mm.³ per hour. With semen to which penicillin had been added immediately after collection and dilution, there appeared to be a similar response to thyroxine, except that the percentage increase in oxygen consumption with thyroxine was less than that to which no penicillin had been added. Increases of from 2 to 12 per cent in the oxygen uptake were obtained with 8 semen samples. The decreased response may be due to the interference by penicillin with oxidative mechanisms of the spermatozoa as well as that of bacteria.

The results obtained in these experiments indicate that total oxygen consumption of spermatozoa is influenced by contaminating substances. However, since washed spermatozoa, as well as unwashed and penicillin-treated semen, showed a similar pattern of response to added thyroxine, it is assumed that spermatozoan metabolism was affected by the treatment.

Wide variability between the oxygen consumption of individual semen samples is influenced by individuality of the semen sample, length of time the semen was stored, the rate of dilution (6) and probably by unavoidable differences in handling individual semen samples.

SUMMARY

Bovine spermatozoa and semen, diluted with a phosphate buffer, were treated with 7 γ per cent DL-thyroxine.

Oxygen consumption generally was increased with the addition of thyroxine in semen samples with an original spermatozoan concentration of from 800,000 to 1,400,000 per mm.³

Semen samples with a spermatozoan concentration of less than 800,000 per mm.³ in the original semen were not influenced on the average by the presence of thyroxine in the concentration used.

The ability of semen to respond with increased respiration rate to added thyroxine appears to be related to some factor that is associated with original spermatozoan concentration.

The influence of bacterial and other contamination on results has been discussed. Its exact influence remains unevaluated.

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RETENTION OF ASCORBIC ACID, CHANGES IN OXIDATION-REDUCTION POTENTIAL, AND THE PREVENTION OF AN OXIDIZED FLAVOR DURING FREEZING PRESERVATION OF MILK

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Outstanding among beverage milk problems is the prevention of the flavor described as oxidized, tallowy or cappy, that tends to develop in milk that is produced under sanitary conditions and has a low bacterial content.

Preservation of milk by freezing is a means of studying the oxidized flavor problem which has been little utilized. In the frozen state, milk is more stable than in the unfrozen state, and its changing characteristics can be studied over a longer period. Babcock *et al.* (1), using samples packaged in paper at a large dairy, found the milk of acceptable beverage quality after storage at -32.8° C. for 115 days. At higher temperatures frozen milk is not as stable and, in the author's experience, always eventually becomes oxidized in flavor. The onset of this defect is a limiting factor in the preservation of milk by freezing and, therefore, is of economic importance.

There are at least four forms of ascorbic acid—two that are levorotatory and are called ascorbic acid and dehydroascorbic acid and two that are dextro-rotatory. The latter two are not biologically active. Of the former, each of which is equally biologically active, ascorbic acid is the only form of vitamin C present in milk in the mammary gland (6). The oxidized flavor ordinarily is detected in milk containing dehydroascorbic acid, the first oxidation product of ascorbic acid, as well as ascorbic acid. Krukovsky and Guthrie (7) concluded that ascorbic acid oxidation is an essential link in the chain of the reactions resulting in the development of the tallowy (oxidized) flavor in milk, and that apparently the oxidation of the lipid fraction of milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic acid and dehydroascorbic acid has been established.

Greenbank (4) believes that the development of an oxidized flavor in milk is related to a change in the oxidation-reduction potential, and that variations in milks can be explained on the basis of differences in their poisoning action.

Dahle and Palmer (3) have expressed the situation as follows: "A condition in the milk which is favorable to the production of the oxidized flavor is apparently favorable to the destruction of vitamin C." They found that the ascorbic acid content gradually decreased during the holding period in all cases, regardless of the development of off-flavor. The reduction in vitamin C was nearly as great in the normal milk as in the milk that developed the off-flavor.

EXPERIMENTAL METHODS

The titration of the milk for reduced ascorbic acid was carried out according

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to the procedure published by Sharp (9). The solution of sodium 2,6-dichlorophenolindophenol was prepared and standardized as described by Stewart and Sharp (11).

In obtaining the milk, no attempt was made to select milk from individual cows, based on their tendency to produce milk that readily developed an oxidized flavor. Morning milk was utilized and was processed in the course of the forenoon. Copper contamination was avoided by employing hand milking and by having the milk delivered in the can into which it was poured from the milk pails.

To delay and prevent the onset of an oxidized flavor, Sharp *et al.* (10) deaerated milk. A convenient laboratory apparatus for deaeration of milk is the one devised by Mottern and Von Loesecke for deaeration of citrus juices (8). This was utilized in the present work.

In deaerating the milk, 12-l. balloon flasks were used and the vacuum was 29 inches. The warm deaerated milk was poured directly into clean well-tinned cans of about 150 ml. capacity, the cans sealed and their contents frozen. Three cold-air storage spaces were utilized: one had a temperature of -10° C., another, -16° C., and the third, -27° C. The temperature of the air in these spaces varied several degrees Fahrenheit. The ascorbic acid was dissolved in a small volume of distilled water and added to the milk.

Unhomogenized milk was found less suitable for these experiments than homogenized milk, since it is less resistant to the onset of the oxidized flavor (13) and, on thawing, shows inferior distribution of insoluble solids. Homogenization was effected at 2,500 lb. pressure per square inch immediately after heating the milk at 62° C. for 30 minutes. The frozen milks were thawed by immersing the cans in warm water.

Oxidation-reduction (E_h) measurements were made with a battery-operated pH meter, using a calomel half-cell. One end of an agar-KCl glass bridge rested in a saturated potassium chloride solution and the other in the sample of milk in a glass beaker. Also partially submerged in the potassium chloride solution was the calomel half-cell and, in the milk was a gold foil electrode fused to a gold wire; these were connected to the jacks of the meter. Each day the electrodes were used, they first were cleaned in the flame of an alcohol lamp, this flame being more suitable than the less pure flame of a Bunsen burner. Each E_h value in the following data is the average of three readings obtained with three electrodes. With but few exceptions, results with the three electrodes were in excellent agreement.

The E_h values of a set of thawed milk samples were obtained either late in the forenoon and again in the afternoon or in the course of the same afternoon but an hour or two apart. In case of differences between the two readings on the same sample, an average value usually was regarded as most representative. The older the samples, the greater this difference was likely to be.

The hand-drawn, uncooled morning milk was processed as follows: *C* (the control)—At the end of the holding period of 30 minutes at 62° C., the milk was homogenized at 2,500 lb. pressure per square inch, cooled, canned and

frozen. *CD*—Similar to *C* except that it was deaerated as already described above its boiling point (50° C.) just before it was canned. *P 20*—Same as the control, except that a solution of ascorbic acid was added to the cooled milk at the rate of 20 mg. per l. of milk. *P 20 D*—Same as *P 20* except that it was deaerated above its boiling point after the addition of the vitamin. *P 40*—Same as *P 20* except that 40 mg. of ascorbic acid per l. of milk was added instead of 20. *P 40 D*—Same as *P 40* except that the warm milk was deaerated after the addition of the vitamin. *20 P*—Same as *P 20* except that the ascorbic acid was added to the raw milk. *20 PD*—Same as *20 P* except for deaeration just before canning. *40 P*—Same as *P 40* except that the vitamin was added to the raw milk. *40 PD*—Same as *40 P* except that the vitamin fortified milk was deaerated before it was canned.

EXPERIMENTAL RESULTS

Figure 1 presents the results of an experiment designed to show the retention at -27° C. of ascorbic acid in milk prepared under various conditions and the

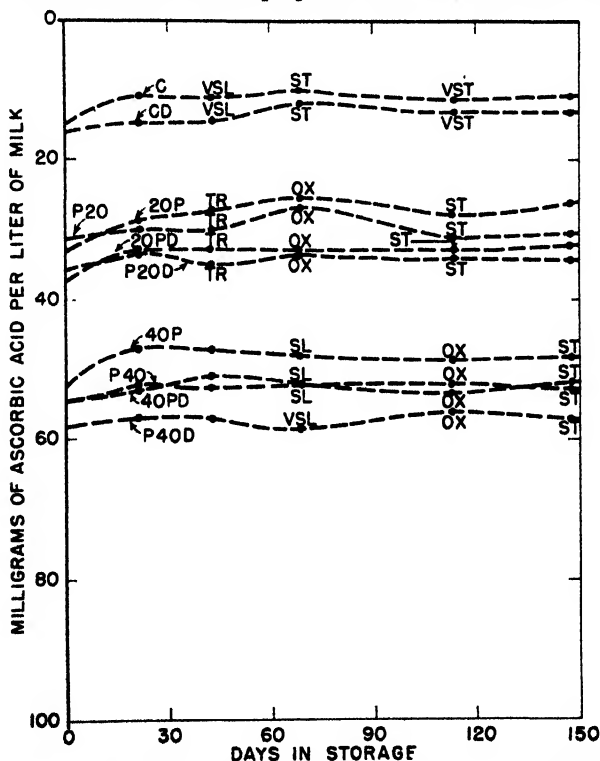


FIG. 1. Retention of ascorbic acid in milk stored at -27° C. and the effect of deaeration and of fortifying the milk with ascorbic acid upon the rate of development of an oxidized flavor. Oxidized flavor intensity is indicated on the curves as follows: tr=trace, v sl=very slight, sl=slight, ox=oxidized, st=strongly oxidized, and v st=very strongly oxidized.

rate of development of an oxidized flavor. To hasten the rate of freezing, these cans of warm milk were immersed in alcohol at -16°C . to which dry ice was added to obtain agitation and to prevent a rise in temperature. Samples were examined after they had been in storage 21, 42, 68, 112 and 147 days. As soon as each fresh milk had been prepared, a small portion was placed in a room maintained at 2 to 4°C . After 9 days, only staleness was detectable in these fluid samples. They were not oxidized.

The ascorbic acid content of the fresh samples was determined 3 to 4 hours after they were placed in the cold room. There was substantially more ascorbic acid in the deaerated than in the undeaerated fresh milks, and this difference was essentially the same in all the thawed samples that were examined during the storage period. Little significance is attached to the apparent advantage of adding ascorbic acid after pasteurization rather than before. Additional evidence on this point is needed.

A straight line drawn from the beginning of each curve to its end would be

TABLE 1
*Reduced ascorbic acid content and E_h of the samples of experiment 2
after 4 days at 2 to 4°C .*

Sample no.	Reduced ascorbic acid (mg./l.)	Oxidation-reduction potential (millivolts)
C	0.0	284
CD	0.0	281
P 20	0.0	289
P 20 D	2.4	278
P 40	4.2	280
P 40 D	13.8	256
P 60	16.2	252
P 60 D	31.8	237

nearly horizontal, especially those lines representing portions of the milk that were fortified with ascorbic acid after pasteurization. Although the concentration of ascorbic acid decreased only slightly, these samples all developed a strong oxidized flavor but at different rates. On a percentage basis, ascorbic acid retention was least in the control and greatest in the deaerated milk that was fortified after pasteurization.

The body of all the thawed samples was good. There was no apparent flakiness or fat separation.

Figures 2, 3, and 4 illustrate the relationship in thawed milk between retention of ascorbic acid, the oxidation-reduction potential and the onset of the oxidized flavor. The morning milk was hand-drawn and, except as indicated, was processed and labeled as in the preceding experiment. All canned samples were in a hardening room before noon for freezing and storage at -16°C . Four days after samples of the freshly prepared milks were placed in the 2 to 4°C . room there was a trace of an oxidized flavor in the control, and the deaerated samples were not as flat in flavor as the non-deaerated ones. Their ascorbic acid content and E_h values are given in table 1.

The reduction in E_h in the freshly prepared samples, as additional units of ascorbic acid were added, is shown in figure 2. The average of several measurements on different milks, in which increments of 25 mg. of ascorbic acid per l. of milk instead of 20 were added, is as follows: first, 25 mg./l., 38 millivolts; second, 25 mg./l., 20 millivolts; third, 25 mg./l., 11 millivolts; and fourth, 25 mg./l., 7 millivolts.

After 23 days in storage, a set of samples was thawed by placing the cans in warm water. Each can was shaken, opened and a portion poured into a small

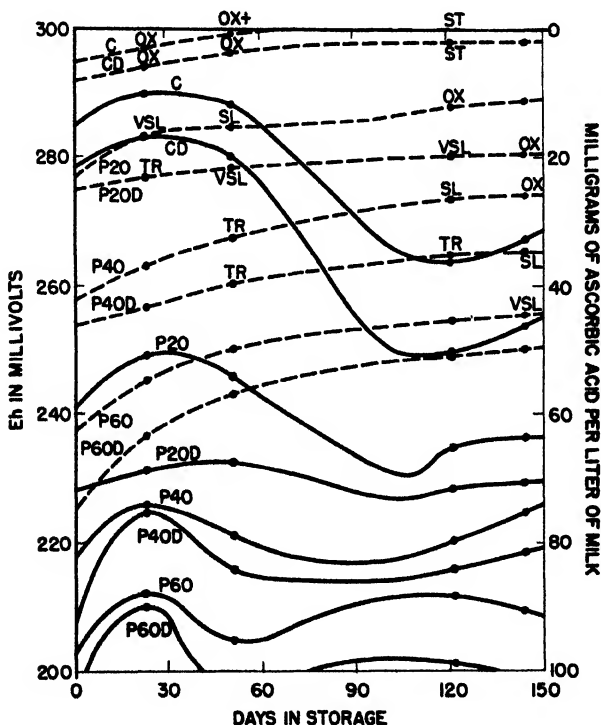


FIG. 2. Retention of ascorbic acid, changes in E_h , and the development of an oxidized flavor in milk stored at -16°C . Broken lines represent mg./l. of ascorbic acid, solid lines the E_h of the samples. (Flavor designations as in fig. 1.)

beaker, after which the opened cans were covered and stored at 2 to 4°C . The beaker samples were warmed to 30°C . and their ascorbic acid content and oxidation-reduction (E_h) values determined. The titrations showed losses in the reducing agent (ascorbic acid), and the E_h measurements disclosed increases in oxidation-reduction potential. Examinations of samples thawed at later dates indicated a continuing decrease in ascorbic acid content but a decrease in E_h followed by an increase. At the same time, an oxidized flavor became detectable and increased in intensity in most of the milks, the rate of increase being greatest

in the control. When a sample which had been fortified by the addition of 60 mg. of ascorbic acid per l. of milk and then deaerated before it was frozen was examined after 160 days in storage, no trace of an oxidized flavor was found. After 51 days in storage, the older these samples were when they were thawed, the greater was the separation of insoluble solids.

Two and 3 days and again 4 to 7 days after each set of thawed milks had been placed at 2 to 4° C., beaker samples were obtained, warmed to 30° C. and the concentration of ascorbic acid and the oxidation-reduction potentials re-determined. These data are plotted in figures 3 and 4. Figure 3 also shows additional

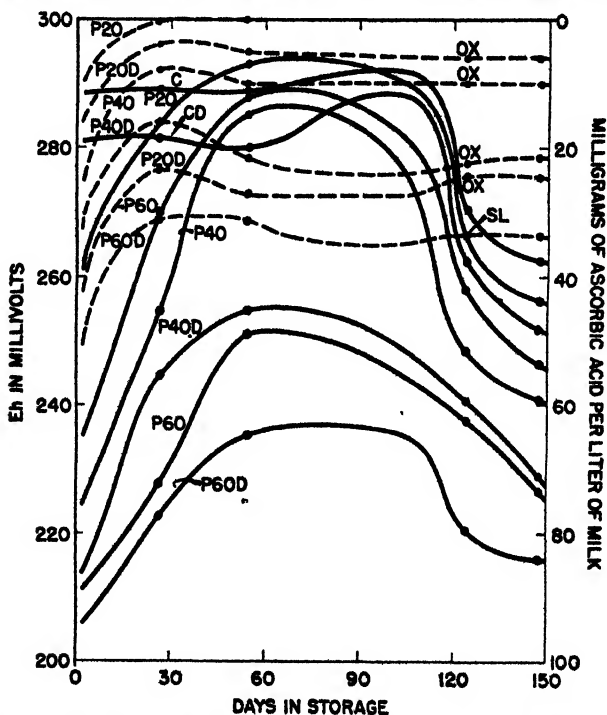


FIG. 3. Ascorbic acid content, E_h and intensity of the oxidized flavor of the thawed samples of figure 2 after 2 to 3 days at 2 to 4° C. (Flavor designations as in fig. 1.)

data on the flavor of the samples. In general, as the amount of ascorbic acid in these thawed refrigerated samples decreased, the oxidation-reduction potential increased, and the intensity of the oxidized flavor increased. A decrease in the intensity of the oxidized flavor never was noted. Having once developed, it always became more pronounced.

Figure 5 shows the effect of the storage temperature upon the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in samples of the same milk containing increasing amounts of added ascorbic acid. Methods used in earlier experiments were followed in preparing

and examining the samples. As soon as the various milks were ready, several cans of each, representing controls and the variables, were placed in compartments maintained at -10 , -16 and -27° C. When 9 days old, a set of refrigerated fluid samples included no milk that tasted oxidized or contained titratable ascorbic acid. The E_h values ranged from 297 to 305 millivolts. Samples stored at -10° C. for 44 days and then thawed were flaky; those held at -16° C. for 98 days also were flaky, but all samples that were thawed after storage at -27° C. were homogeneous. As in other experiments with but few exceptions, the more

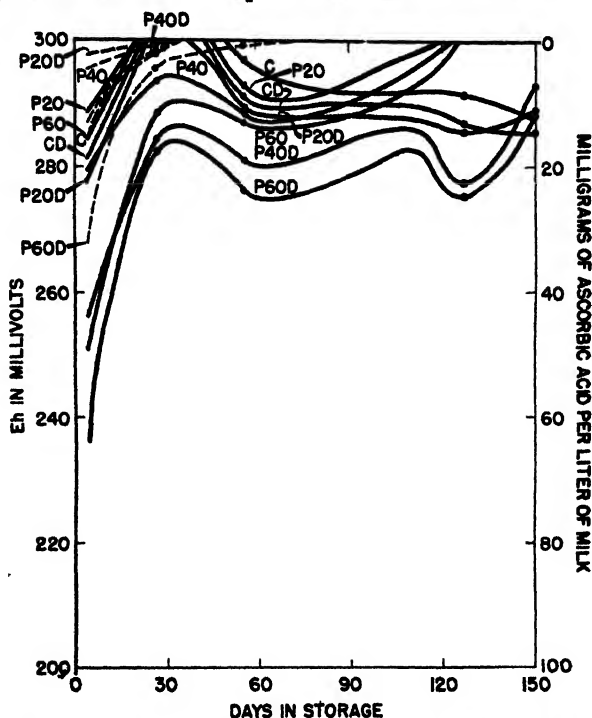


FIG. 4. Ascorbic acid content and E_h of the thawed samples of fig. 2 after 4 to 7 days at 3 to 4° C.

the milk was fortified with ascorbic acid and the lower its storage temperature, the greater was the resistance of the milk to the onset of an oxidized flavor.

The evidence in figure 5 indicates that oxidation of ascorbic acid and the onset of the oxidized flavor in milk may be accompanied by a reduction in the electromotive force or chemical energy of the system. After 98 days in storage, during which there was a loss in ascorbic acid and the development of an oxidized flavor, most of the samples had a lower oxidation-reduction potential than they did when they were frozen.

DISCUSSION

Many who have referred to the off-flavor known as "oxidized" or "tallowy"

have taken it for granted that oxidation of the fat was the cause. The view that the true fat is involved is unsound. Milk fat is too stable to be affected by this mild reaction (5). There is considerable evidence that a phospholipid, a relatively unstable fat-like substance containing phosphorus and associated with the fat, is the constituent of milk that is affected.

Although oxidation of a phospholipid, probably lecithin, now quite generally is regarded as the cause of the defect, oxidation-reduction potential measurements have been used by only a relatively few investigators in studying the problem. This paper attempts to show oxidation-reduction changes during the onset of the off-flavor in frozen milk by examining its thawed product, and at the same time recording the decrease in ascorbic acid.

In the experiment on which figure 1 is based, it was demonstrated that a

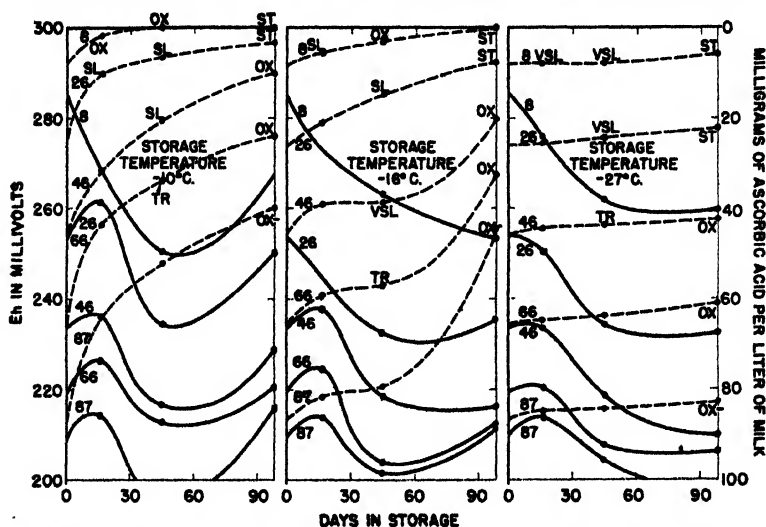


FIG. 5. Relationship between the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in milk stored at different temperatures. The corresponding ascorbic acid and E_h curves are indicated by numbers which also represent the mg. per l. of ascorbic acid in the fresh samples. Broken lines represent ascorbic acid, solid lines oxidation-reduction potential (E_h) of the samples. (Flavor designations as in fig. 1.)

strong oxidized flavor may develop in milk with but slight decrease in ascorbic acid content; also, the rate of development is much slower in milk that has been fortified with the acid. Deaeration aided in the preservation of the acid but only slightly retarded the onset of the off-flavor.

The storage temperature in the second experiment was not as cold as in the first; figure 2 shows that the retention of ascorbic acid was not as effective. Here again, fortification with ascorbic acid protected the fresh flavor of the milk. Although the E_h values, relative to their initial relationships, continued to follow approximately the same pattern, they did not continue to increase but rather decreased and then began to increase again.

Insofar as these data are concerned, it appears that during storage frozen milk may become more oxidized in flavor while its oxidation-reduction potential is decreasing. Whether or not the E_h values of the thawed samples at 30° C. accurately reflect the E_h values during frozen storage is not known. If they do, then opposing reactions were going on during a large part of the storage period. One reaction is a slow oxidation of ascorbic acid to dehydroascorbic acid and the other (or others) is reducing in character and of greater effect upon the oxidation-reduction potential of the system. If E_h values after thawing do not reflect the E_h values during storage, then constituents redissolved and soon approached a new equilibrium that substantially altered the oxidation-reduction potential. Dahle, *et al.* (2), working with frozen cream, obtained results that resemble these on frozen milk.

In the present study all E_h measurements were made in the same manner, and no reading was acceptable if there was drifting and the electrodes were not in agreement. The data, therefore, should be relative.

Swanson and Sommer (12) conclude from their studies on oxidation-reduction potentials in relation to the development of oxidized flavor in fluid milk that the E_h value of the medium does not seem to inhibit or accelerate the development of the off-flavor.

CONCLUSIONS

From the data of this paper, it may be concluded that a low E_h , obtained by adding ascorbic acid, greatly defers but does not prevent the development of an oxidized flavor in frozen milk. However, a low E_h does not increase the retention of vitamin C in the form of ascorbic acid.

In determining the flavor of the milks, the author had the benefit of the experienced judgment of C. J. Babcock.

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LIPID DETERIORATION IN DAIRY PRODUCTS. THE STABILITY OF MILK FAT AND FAT-SOLUBLE VITAMINS AS DETERMINED BY THE RE-EMULSIFICATION TEST

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The milk excreted by the mammary gland under proper sanitary and feeding conditions has a pleasant and sweet taste. Quite often, however, milk and its products develop flavors which render them distasteful to the human palate and are the cause of their rejection. There are rancid and oxidized flavors. While it is possible to reduce to a minimum, retard or prevent the lipolysis by a quick and prompt cooling of raw milk, storage at constant temperatures (2, 8) and subsequent pasteurization treatments (9), uniform control of the development of oxidized flavors is not possible.

The development of oxidized flavors in fresh milk could be practically retarded or prevented by the deaeration of milk (11), or depletion of milk of its total vitamin C content by rapid oxidative methods (6, 7). The retardation of oxidized flavors by deaeration and their stimulation in the presence of dissolved oxygen and vitamin C by the exposure of fresh milk to sunlight for a short period of time or by copper catalysis of the reaction (6, 7), indicates that oxygen plays an important part in the reaction which produces the oxidized flavors, and that the reaction is promoted by a catalyst. The prevention of the oxidized flavors by the complete depletion of the total vitamin C content of milk, irrespective of the oxygen and copper present, suggests that oxygen and copper, either alone or together, could not promote the reaction which produces the oxidized flavors (6, 7).

Thus, to develop the oxidized flavors in fresh milk, all components of the system must be present, namely, oxygen, vitamin C, catalysts such as copper, light or enzymes, oxidation-susceptible lipid fraction of the milk or, as recently reported, casein (13).

It long has been believed that vitamin C is an anti-oxidant, and that if it were increased in the milk, the development of the oxidized flavors would be prevented (1, 11, 12). Under certain conditions, vitamin C might exert a protective influence. However, the amounts of added ascorbic acid required for protection vary from sample to sample. Undoubtedly, this is due to the variation in the amounts of dissolved oxygen in the samples of milk. It has been shown that partial and quick oxidation of ascorbic acid in milk to dehydroascorbic acid, either by added hydrogen peroxide or by oxygen with light as a catalyst, brings the system more quickly to the point where the other reactions which produce the oxidized flavor in milk could be coupled to that involving ascorbic acid oxidation (6, 7). Consequently, the amount of ascorbic acid added to milk must be considerably in excess of that required to utilize all of the dissolved oxygen prior to

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the establishment of conditions favorable to the secondary reactions which result in the development of the oxidized flavor. The differences between samples of milk fortified with ascorbic acid in their abilities to resist the reaction which produces the oxidized flavor also could be attributed to variations in the ability of milk to promote ascorbic acid oxidation.

Ascorbic acid in milk is oxidized to dehydroascorbic acid which is a very unstable form of vitamin C and is readily destroyed by heat. Its accumulation in the milk is governed primarily by the rate of ascorbic acid oxidation. When dehydroascorbic acid is destroyed as rapidly as it is formed by the oxygenation of milk during pasteurization, the secondary reaction which produces the oxidized flavor is prevented. In this connection, it should be noted that the development of oxidized flavors in fresh milk apparently has nothing to do with the deterioration of milk fat, which is relatively stable. However, milk fat itself also undergoes oxidative deterioration in the presence of ascorbic acid, resulting in the development of metallic to fishy flavors. These flavors have been traced to oxidative processes which occur in the milk fat at the end of its storage life. Quite often they are accompanied by the extensive losses in vitamins A, E and carotene content of the milk fat. The susceptibility of milk fat to this type of deterioration depends on the type of product, the temperature of pasteurization and the conditions of storage. These factors were studied in some detail, and the data are presented to show that under certain conditions, namely, at the end of storage life of the milk fat, ascorbic acid plays an important part in the oxidative deterioration of fat, manifesting itself by the development of the objectionable oxidized flavors and the losses in the fat-soluble vitamins.

EXPERIMENTAL

In a recent study of the oxidation of vitamin A and its precursor, carotene, in milk fat (4, 5), it has been pointed out that the reemulsification test was found to be very useful in recognizing not only the flavor defects of milk fat, but also in detecting changes in the resistance of milk fat to oxidation, whether these changes are brought about during storage, by exposure to light, or any other factor. Consequently, in order to study the effects of different factors upon the ability of milk fat to resist deterioration in the presence of ascorbic acid, the following procedure was adopted.

At the end of various storage periods, milk fat, held either in the form of cream¹, butter¹ or pure fat¹, was re-emulsified in skim milk² depleted of its total vitamin C content by rapid oxidative method (7) to produce 4 per cent fat re-constituted milk. To aliquot portions of this milk, approximately 20 mg. of ascorbic acid and 0.1 mg. of copper per liter of milk were added, either alone or together. These portions of milk then were held at 0 to 5° C. up to 48 hours. Throughout the duration of the experiments, the samples were protected from

¹ Milk fats were obtained by churning of cream, oiling and centrifuging of butters prior to their use in the re-emulsification test, or prior to storage.

² In Club aluminum cream maker. The skim milk was depleted of its total vitamin C content by added H_2O_2 and the following pasteurization at 61.6° C (143° F) for 30 minutes (7).

TABLE 1

The effects of complete depletion of natural milk of its total vitamin C content by the oxygenation of milk during pasteurization upon the development of oxidized flavor in milk subsequently held at 0 to 5° C.

Held at 0-5° C.	Untreated control milk		(B) Oxygenated milk		Additions to (B) milk	
Days	Ascorbic acid	Flavor scores	Total vit. C	Flavor scores	Ascorbic acid	Flavor scores
	(mg./l.)		(mg./l.)		(mg./l.)	
0	12.3		00.0		20.8	
1	6.8	38 T		42	13.0	42
2	0.0	25 T	.	42	3.0	38
3	..	Ex. T.	.	42	0.0	25 T
Copper added—0.1 p.p.m.						
1	00.0	25 T		42	1.9	25 T
12		Ex. T.		42		Ex. T

light. They were scored for flavors; then the gravity cream was churned, and the butter obtained was centrifuged and the fat was analyzed for its fat soluble vitamin content. The judging was done on the basis of the score card recently revised by the American Dairy Science Association². Vitamins A and E and peroxides were determined, using Koehen and Sherman (3), Quaife (10) and Volz and Gortner (14) methods, respectively.

It already has been remarked that when dehydroascorbic acid is destroyed as rapidly as it is formed, the secondary reaction which produces the oxidized flavors in milk is prevented. To test this point, a batch of fresh mixed milk was oxygenated during the pasteurization at 61.6° C. for 30 minutes. The oxygen was admitted in minute bubbles into milk undergoing treatment, using carborundum gas diffusion tubes.

TABLE 2

The effects of complete depletion of natural milk of its total vitamin C content by hydrogen peroxide and the following pasteurization and of added ascorbic acid and copper upon the development of oxidized flavors and the stability of fat soluble vitamins in milk subsequently held at 0 to 5° C.

Additions			Flavor scores of milk				At the end of 14 days holding period		
Ascorbic acid	Cop- per	Ascorbic acids ^a	Hours at 0 to 5° C.				Per 100 g. fat		
			3	6	9	14	Carot.	Vit. A	Vit. E
(mg./l.)	(mg./l.)	(mg./l.)					(μg.)	(μg.)	(μg.)
..	40	40	40	40	438	421	2125
..	0.1	..	40	40	40	40	433	419	2131
20.2	..	20.9	40—	30MT	25MT	Ex.MT	432	401	2183
20.2	0.1	20.9	25MT	25MT	Ex.M.	Ex.M.	432	399	2071

^a Ascorbic acid re-added at the end of a 6 day storage period.

² Flavor scoring system: 40, no criticism; 35-40, acceptable to some consumers; 25, unsuitable for consumption. Symbols: Ex. = extremely; T. = tallowy; M. = metallic; F. = fishy; Oi. = oily.

The data presented in table 1 are rather conclusive in showing that the depletion of milk of its total vitamin C content by oxygenation prevents the development of the oxidized flavors, and that the oxidized flavors could be induced again by the addition of ascorbic acid to oxygenated milk. The results of this experiment are in agreement with the original one (6, 7).

The data of table 2 show that, although the oxidized flavors developed in samples of fresh milk containing added ascorbic acid prior to storage at 0 to 5° C., the vitamins A and E and carotenoid content of the fat remained practically unaffected. In this case, a sample of fresh mixed milk was first depleted of its total vitamin C content by hydrogen peroxide and the following pasteurization at 61.6° C. for 30 minutes.

The data presented in table 3 show the effects of the addition of ascorbic acid

TABLE 3

The effects of the addition of ascorbic acid and copper to reconstituted milks made of fresh skim milk depleted of its total vitamin C content or of skim milk powder and unstable fat (re-emulsification test) upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconstituted milk—4% fat							
Made of	Additions		Flavor scores of milk		Per 100 g. of fat		
	Ascorbic acid	Copper	Hours at 0 to 5° C.		Carotenoids	Vitamins	
			24	48		A	E
	(mg./l.)	(mg./l.)			(μg.)	(μg.)	(μg.)
fresh skim milk	Control fat		40-oi	40-oi	671	498	2062
		.1			640	441	1889
	20.7		Extremely		416	308	1234
	20.7	.1	metallic and fishy		395	269	1127
skim milk powder			40-	40-	599	438	2165
		.1	40-	40-	610	428	2018
	19.7		25 F	25 F	410	401	1074
	19.7	.1	Extremely metallic and fishy		380	305	1096

and copper to reconstituted milk (re-emulsification test) made of fresh skim milk depleted of its total vitamin C content, or of skim milk powder, and of fat susceptible to deterioration, upon the development of oxidized flavors and losses of the fat soluble vitamins. The results showed that extreme metallic to fishy flavors developed in samples of milk containing ascorbic acid added either alone or together with copper. The development of these flavors was also accompanied by considerable losses in vitamins A and E and carotenoid content of the fat.

These observations indicate, therefore, that the oxidized flavors in fresh milk are not associated with the milk fat, but with the unstable materials present either on the surface of the fat globules or in the plasma phase of the milk. This is clearly evident from a comparison of the data of tables 2 and 3.

Subsequently, it was thought of importance to obtain some idea concerning the effects of the following factors upon the development of the objectionable flavors and losses in the vitamins A and E and carotenoid content of the milk

fat in the re-emulsification test: the fat content of reconstituted milk, the amounts of added ascorbic acid, the exposure to light of fats susceptible and non-susceptible to oxidative deterioration, and the temperature of pasteurization of skim milk used in the test.

The data presented in table 4 show that in the re-emulsification test the development of the objectionable flavors was not retarded, and the losses in fat-soluble vitamins were not appreciably affected, either by the fat content or by the vitamin C content of the reconstituted milk products.

The data on the effect of the exposure of fat to light are presented in table 5. This experiment was prompted by the previous observations (4, 5), indicating

TABLE 4

The effects of the fat content of reconstituted milk (re-emulsification test) and of the amounts of subsequently added ascorbic acid upon the development of oxidized flavors and losses of the fat soluble vitamins

Type of fat (A)	Reconstituted milk made of skim milk and fat (A)								
	Fat content	Additions		Flavor scores		Per 100 g. fat			
		Ascorbic acid	Copper	hours at 0 to 5° C.		Carotenoids	Vitamins		Per-oxides
				3	24		A	E	
	(%)	(mg./l.)	(mg./l.)	(butm.)		(μg.)	(μg.)	(μg.)	(Milk-equiv.)
Suscept. to oxidative deterioration	Con.	fat	628	500	1909	0.516
	4.0	---	..	40--	40	612	482	1528	0.431
	4.0	..	.1	40--	40	630	462	1737	0.397
	4.0	23.0	..	25T	Ex.F.	554	354	1084	0.368
	4.0	23.0	.1	Ex.FT	Ex.FT	401	268	785	0.497
	4.0	106.3	..	25T	Ex.FT	559	405	1265	0.371
	4.0	106.3	.1	Ex.FT	Ex.FT	331	245	721	1.116
	20.0	40--	40	594	480	2082	0.453
	20.01	40--	40	639	491	1714	0.511
	20.0	23.5	..	Ex.FT	Ex.FT	564	412	1187	0.505
	20.0	23.5	.1	Ex.FT	Ex.FT	493	328	970	0.880
	20.0	82.1	..	Ex.FM	Ex.FM	568	398	1403	0.354
	20.0	82.1	.1	Ex.FM	Ex.FM	420	359	1004	0.692

that vitamin A in the milk fat is readily photo-oxidized, whereas its precursor, carotene, remains unaffected. It was of interest, therefore, to find out also the effects of irradiation of stable and unstable fats upon the stability of tocopherols, and the susceptibility of fat to deterioration in the re-emulsification test. The irradiation experiment was performed following the same procedure as previously described (4, 5) with the exception that the temperature of the fat was maintained at 45 to 48° C. and the intensity of the light generated by the mercury vapor lamp at approximately 1400 foot-candles. The data in table 5, although not directly comparable, revealed that the susceptibility of fat to the foregoing type of deterioration depends primarily upon its ability to resist oxidation prior to irradiation, and to lesser degree upon the direct effect of irradiation. The data in table 5 also indicate that, irrespective of the ability of fat to

resist oxidation in the re-emulsification test, vitamin A was found to be photo-oxidized at a much faster rate than vitamin E, whereas the carotenoid content of the fat remained practically unchanged throughout the duration of the irradiation. Subsequently, it was thought of importance to obtain some idea concerning the effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of the objectionable flavors and losses in the vitamins A and E and the carotenoid content of the milk fat. For this reason, ascorbic acid and copper were added either alone or together to portions of reconstituted milks made of unstable fat and of fresh skim milks depleted of

TABLE 5

The effects of irradiation of fats susceptible and non-susceptible to deterioration with light generated by mercury vapor lamp upon the fat soluble vitamin content, and the susceptibility of fat to oxidative deterioration as determined by the re-emulsification test

Reconstituted milk made of skimmilk and fat (A)							
Minutes irradiat. at 45-48° C.	Type of fat (A)	Additions		Flavor scores hours at 0 to 5° C.	Per 100 g. fat		
		Ascorbic acid	Copper		Carot-enoids	Vitamins	
		(mg./l.)	(mg./l.)		(μg.)	(μg.)	(μg.)
Control	Suscept.	Control fat			634	492	2047
	"		.1	40-Oi	602	416	1670
	"	20.0	...	fishy	387	316	1026
	"	20.0	.1	ex. F.	204	186	906
60 minutes	Suscept.	Control fat			585	160	1540
	"		.1	40-Oi	553	167	1396
	"	20.0	...	fishy	443	116	1164
	"	20.0	.1	ex. F.	229	73	1134
Control	Non-susceptible	Control fat			1024	883	3917
		average all sam.		40	1019	872	3966
60 minutes	"	Control fat			1026	308	3777
	"		.1	40-Oi	1015	240	3971
	"	24.3	...	39-Oi	1010	224	3840
	"	24.3	.1	35-OiMT	971	201	2809

their total vitamin C content by added hydrogen peroxide, and the following pasteurization at 61.6 and 76.6° C. for 30 minutes.

A comparison of the data in table 6 suggests the possibility that the inactivation of a catalyst-enzyme by heat primarily was responsible for the retardation of inter-action between ascorbic acid and fat or fat-soluble vitamins in the milk, which was made of skim milk pasteurized at 76.6° C.

Although the evidence presented in the preceding paragraphs definitely indicates that the oxidative deterioration of milk fat could be catalyzed in the presence of ascorbic acid, nevertheless, it was apparent that the reaction might take place only at the end of the storage life of fat. Since it seemed possible that the changes in the ability of milk fat to resist the foregoing type of deterioration would be affected by the type of product held, the temperature of pasteurization

TABLE 6

The effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconst. milk made of skim milk and unstable fat				Per 100 g. of fat				
Additions		Flavor scores		Carot- enoids	Vitamins		Perox- ides	
Ascor- bic acid	Cop- per	Hours at 0 to 5° C.			A	E		
		1 & 24	48					
(mg./ l.)	(mg./ l.)	(Skm.) ^a	(Butm.) ^a	(μg.)	(μg.)	(μg.)	(milli- equiv.)	
Prior to re-emulsification				506	452	2431	0.196	
After re-emulsification in the skim milks:								
(I). pasteurized at 61.6° C.								
control		40	40	495	442	2197	.153	
	.1	40	40	483	432	2080	.202	
19.7	25F	25F	Ex. F.	407	399	1808	.356	
19.7	.1	Ex. F.	Ex. F.	327	330	1294	.470	
(II). pasteurized at 76.6° C.								
control		40	40	473	436	2475	0.28	
	.1	40	40	501	444	2284	0.18	
22.1		40	30F	477	451	2121	0.22	
22.1	.1	40	35T	Ex. F.	449	389	1468	0.20

* Skm., gravity skim milk; Butm., buttermilk after the churning of gravity cream.

zation and the condition of storage, it was thought desirable to determine what effects these factors might have upon the storage life of fat as determined by the re-emulsification test.

For this reason, four lots of cream, butter and pure fat were prepared from mixed morning milks obtained from the Cornell University herd. Two portions of this milk were oxygenated continually during the pasteurization at 61.6 and 76.6° C. for 30 minutes. This was done to deplete the milk of the total vitamin C content prior to preparation and storage of the previously described milk products. The other two portions of the same milk were pasteurized at the same

TABLE 7

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, and the temperature of pasteurization upon the development of the tallowy flavor in cream during its storage at -17.7 to -16.1° C.

Milk		Flavor scores of cream and its buttermilk at the end of storage at -17.7 to -16.1° C.							
Treatment and Pasteurized at	Product	month							
		3	4	5	6	7	8	10	12
61.6° C. control	Cream	38T	39T	39T	40	39T	38T	38T	30T
	Buttermilk	38T	39T	40	40	39T	38T	40	35T
76.6° C. control	Cream	40	40	40	38T	25T	25T	25T	25T
	Buttermilk	38T	38T	39	ExTM	ExTM	ExTM	ExTM	ExTM
61.6 and 76.6° C. oxygenation	Cream and Buttermilk	40	40	40	40	40	40	40	40

temperatures, but without the oxygenation treatment (tables 7 and 8). In another experiment, a portion of mixed milk was first depleted of its ascorbic acid content by adding hydrogen peroxide. The aliquot portions of each cream, separated from the control and ascorbic acid depleted milks, then were pasteurized at 61.6, 68.3, 71.1 and 76.6° C. for 30 minutes prior to churning and storage of cream, butter and pure fat (table 9). Throughout the duration of the experiments, the samples of cream and fat were held in tightly sealed glass containers protected from the light. The butter samples were shaped in cylindrical forms and wrapped directly in two thicknesses of tinfoil. The samples of cream, butter and pure fat subsequently were held at -17.7 to -16.1° C.

The data presented in table 7 show that the creams separated from milks

TABLE 8

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, the temperature of pasteurization and storage of cream, butter and fat at -17.7 to -16.1° C upon the susceptibility of fat to oxidative deterioration (storage life of fat) as determined by the re-emulsification test.

Milk		Product held	The end of the storage life of fat
Pasteurized at	Treatment		
(°C.)			(months)
61.6	control	cream	4
	oxygen.	cream	4 to 5
76.6	control	cream	5
	oxygen.	cream	6
61.6	control	butter	4 to 5
	oxygen.	butter	5 to 6
76.6	control	butter	not sensitive to deterioration at the end of 12 months
	oxygen.	butter	
all temperatures	control & oxygen.	fat	"

Storage cream—56 to 58 per cent fat.

depleted of their total vitamin C content by oxygenation during pasteurization were found by judges to be perfect in flavor at the end of 12 months storage at -17.7 to -16.1° C. Moreover, it was found that the depletion of cream of its total vitamin C content by added hydrogen peroxide, and the following pasteurization at indicated temperatures resulted in the prevention of oxidized flavors, even at the end of 2 years storage at -17.7 to -16.1° C. It should be noted, however, that these creams developed a slightly "nutty" flavor at the surface, whereas the control samples of cream developed very strong tallowy flavor.

The data in tables 8 and 9 reveal that the temperature of pasteurization of milk or cream and the type of product held are the factors governing the susceptibility of fat to oxidative deterioration in the presence of added ascorbic acid. They also show that, irrespective of the temperature of pasteurization, the milk fat stored in the form of cream loses its resistance to oxidative deterioration at a much faster rate than the fat stored in the form of butter; and that only the

fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat were found to be non-susceptible to oxidation resulting in the development of the objectionable flavors when re-emulsified in skim milk at the end of 1- and 2-year storage periods.

The re-emulsification test was found to be extremely useful in determining the sensitivity of fat to oxidation when the fat was obtained from products which as yet have not shown any apparent changes either in their flavors or in their fat soluble vitamin content. This is clearly evident from a comparison of data of tables 7 and 8. It shows that the cream separated from oxygenated milk did not develop any "off" flavors during the 12-month holding period. This period, during which neither organoleptic nor chemical changes could be accurately determined, often is referred to as the storage life of fat or of a food product. Yet, in the presence of ascorbic acid, with or without copper, the fat undergoes deterioration which is manifested by the development of the objectionable flavors and the losses of fat-soluble vitamins. This would explain the development of the distasteful flavors in food products made of storage cream plus products containing vitamin C, such as fresh milk or lemon juice.

In conclusion, it is of importance to point out that the development of "off" flavors in milk, as a result of ascorbic acid oxidation with copper as a catalyst, might not necessarily be connected with the deterioration of milk lipids. This was evident from the fact that occasionally a flavor which is difficult to describe developed at the end of a 48-hour storage period in the skim milk used as a control sample in the re-emulsification test. This would be in line with the experimental results obtained by Thompson *et al.* (13).

Although this flavor might be present in the gravity skim milk obtained from the reconstituted milk, it seldom was detected in the gravity cream butter-milk. This suggests that either some factor, unknown at present, prevents the development of this flavor in the gravity cream, or that the substance responsible for it was denaturated during the churning of cream.

Usually no objectionable flavors develop in the gravity cream buttermilk when fat used in the re-emulsification test is stable. The metallic to fishy flavor developed in reconstituted milk made of oxidation-sensitive fat and containing ascorbic acid, either alone or with copper, quite often was intensified by the churning of gravity cream. This flavor does not develop in the presence of copper alone.

The oxidized flavors which developed in the control samples of milk products during their storage might be carried into the portion of reconstituted milk containing copper alone. The churning of gravity cream from such milk may not result necessarily in the intensification of this flavor.

SUMMARY

(1) It has been shown that ascorbic acid plays an important part in the oxidative deterioration of milk fat at the end of its storage life, as determined by the re-emulsification test, resulting in the development of objectionable

flavors and losses in vitamins A and E and the carotene content of the fat. The susceptibility of fat to this type of deterioration is determined primarily by the treatment of milk, the temperature of pasteurization, the type of product, the conditions of storage, and to a lesser extent upon the direct and immediate effect of the exposure to light.

(2) The exposure of pure fat to light generated by mercury vapor lamp (1400 foot-candles) slightly affects its vitamin E content. However, it lowers the resistance of vitamin E in the stable fat to deterioration as determined by the re-emulsification test.

(3) The re-emulsification test was found to be useful in determining the end of the storage life of fat when the fat was obtained from products which have not as yet shown any apparent changes in their flavors. This view is supported by the observations showing that the depletion of cream of its total vitamin C content, either by oxygenation, or by hydrogen peroxide, has prevented the development of the objectionable flavors for 12 and 24 months at -17.7 to -16.1° C., respectively. In the re-emulsification test, however, the fat obtained from oxygenated milk pasteurized up to 76.6° C. lost its ability to resist the foregoing type of deterioration at the end of 4 to 6 months of storage, depending upon the conditions of processing.

(4) Only the fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat retained their abilities to resist deterioration in the re-emulsification test at the end of two years storage at -17.7 to -16.1° C.

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LACTATING FACTORS FOR DAIRY COWS IN DRIED GRAPEFRUIT PEEL

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Recent reports by Huffman et al. (2, 3) have shown that dairy cows fed alfalfa alone declined abnormally in milk production. A marked increase was obtained by supplementing the alfalfa with corn silage (2) or beet pulp or corn gluten meal (3). These feeds were claimed to contain unknown milk stimulating factors. Because of the interest throughout the citrus-producing states in the use of dehydrated citrus by-products in dairy rations (1, 4, 5), experimental work was conducted to ascertain the value of dried grapefruit peel as a source of these milk stimulating factors.

EXPERIMENTAL PROCEDURE

Two sources of dried grapefruit peel¹ were used in the feeding trials. One was sun-dried in the open desert. No juice was removed from the peel previous to drying. The other was dried mechanically. Part of the juice was removed before dehydration.

Four pairs of cows were placed into two lots. One of each pair eventually received sun-dried peel and its mate mechanically dried peel. Each pair was identical in breed and nearly identical in period of lactation and age. Numbers 56, 57, 60 and 61, Guernseys, were fresh, 40, 42, 16 and 17 days, respectively, when placed on experiment; 158 and 159, Jerseys, 41 days; and 257 and 272, Holsteins, 50 and 33 days, respectively. Numbers 60, 61 and 257 were first-calf heifers and the remainder were second-calf heifers.

As soon as the cows were placed on experiment, they were fed first-cutting alfalfa hay ad libitum in dry lot until milk production markedly decreased. This period was of 6 weeks duration for 158, 159 and 272 and 9 weeks for the remaining five animals. At the end of this period, the alfalfa was supplemented with 2 lb. of dried grapefruit peel twice daily for each cow for 4- to 5-week periods. After this, the dried citrus was replaced with an equal amount of a grain mixture consisting of six parts barley, six parts wheat bran, two parts cottonseed meal and two parts beet pulp. After 4 weeks on this ration they were supplemented with oat pasture. Four lb. of the grain mixture furnished approximately 2.91 therms of energy and 4 lb. of the dried grapefruit peel approximately 2.98 therms.

RESULTS AND DISCUSSION

The milk production records are given in table 1. In every case there was a decline in milk production during the alfalfa feeding period. When supple-

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TABLE 1

Effect of dried grapefruit peel on milk production (Av. daily production during test week)

Feeding period	Sundried grapefruit peel fed			Mechanically dried grapefruit peel fed			
	Week of period	Guernseys (av. of 56 & 60)	Jersey 159	Holstein 257	Guernseys (av. of 57 & 61)	Jersey 158	Holstein 272
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Alfalfa alone (6-9 weeks)	First	30.7	25.4	36.7	31.1	33.3	43.4
	Last	20.9	15.7	26.7	19.7	19.0	19.4
Alfalfa + 4 lb. citrus daily (4-5 weeks)	First	22.2	16.7	28.7	20.9	18.5	23.4
	Last	22.4	15.6	29.2	20.8	18.5	20.9
	Av.	22.4	16.1	29.0	20.8	18.2	21.7
Alfalfa + 4 lb. grain mixture daily (4 weeks)	First	21.4	15.5	27.0	19.9	18.4	17.5
	Last	20.5	13.6	23.6	17.4	15.3	16.4
	Av.	20.4	15.0	25.4	19.0	16.9	17.5
Alfalfa + grain mixture + pasture (3 weeks)	First	21.8	13.4	25.3	20.7	17.5	
	Last	23.0	13.9	27.3	21.4	17.2	
	Av.	22.6	13.8	26.0	21.4	17.3	

ments of either source of dried grapefruit peel were given, there was a small but definite increase in milk production for seven of the eight cows. In the case of Jersey cow 158, the dried peel only lessened the decline caused by the alfalfa ration. However, milk production remained constant for this cow during the 5-week period in which the peel was fed. There was no significant difference between the results received with either of the two sources of dried grapefruit peel.

In order to eliminate the effect of energy in the dried grapefruit peel upon milk production, the dried peel was removed and an equal amount of grain mixture given. This grain mixture was approximate in energy to the dried grapefruit peel. Milk production definitely fell off in the first week of this period except for the two Jerseys. During this total period of 4 weeks, there was a definite decline in milk production for all the cows. This proved that the increase caused by the dried peel was not caused by added energy. When the cattle were

TABLE 2

Weight records of cows

Period of feeding	Weight of cow no.							
	56	60	159	257	57	61	158	272
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Alfalfa alone								
At start	973	908	885	1183	942	780	843	1443
At end	982	853	873	1132	973	752	798	1490
End of alf. + 4 lb. citrus	985	878	890	1107	912	755	812	1527

placed on pasture there was a definite increase in milk production except for Jersey 159.

The weight records of the cows are given in table 2. During the alfalfa feeding period, there was no significant change in weight. Three of the cows gained weight, four lost weight and one remained the same. During the period of citrus feeding four of the cows definitely gained weight, two remained the same and two lost weight.

From the above results, it is apparent that both sundried and mechanically dried grapefruit peel contain the unknown milk-stimulating factors first demonstrated in certain feeds by Huffman et al. (2, 3). The grain mixture fed did not contain these factors in appreciable amounts, while oat pasture did. The findings give emphasis to the value of dehydrated citrus products in dairy rations.

SUMMARY

Four lb. daily of dried grapefruit peel added to an alfalfa hay ration increased milk production. An equal amount of a grain mixture did not maintain this increase. Supplementing a ration of alfalfa hay and concentrate mixture with oat pasture definitely increased milk production. It is concluded that dried grapefruit peel contains factors which stimulate milk production in dairy cows.

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THE NUTRITION OF THE NEWBORN DAIRY CALF

III. THE RESPONSE TO A PHOTOLYZED MILK DIET

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A series of reports from the Illinois Agricultural Experiment Station recently have contributed significantly to the fundamental information on the nutrition of the very young calf. Wiese *et al.* (17) prepared a "synthetic milk" which resulted in normal growth when fed to calves from 48 hours to 12 weeks of age. When riboflavin was omitted from this "synthetic milk", deficiency symptoms developed in from 2 to 6 weeks (18). Since these experiments have shown that the very young calf requires a dietary source of riboflavin, it was of interest to study the effects of feeding natural milk in which a major portion of the riboflavin had been destroyed.

Numerous reports in the literature (4, 5, 7, 13, 14, 19) have shown that significant amounts of the riboflavin of milk are destroyed by exposure to sunlight. Preliminary work showed that under proper conditions of sunlight exposure sufficient quantities of the riboflavin in colostrum and milk could be destroyed to yield a product which was extremely low in this vitamin. Studies then were begun to determine the effect of feeding photolyzed colostrum and milk to newborn or very young calves.

EXPERIMENTAL

Four purebred, male, Guernsey calves were used in this experiment. Two of these calves (*A* and *B*) were taken from their dams at birth and placed in 6 x 6-foot wooden box stalls with wire mesh flooring. Calf *A* was bedded with straw. All subsequent calves were bedded on burlap sacks to eliminate the possibility of the ingestion of straw stimulating rumen synthesis of riboflavin. Calves *A* and *B* were fed for 3 days on colostrum which had been photolyzed previously, frozen and stored¹ for over 3 weeks. The two remaining calves, *C* and *R*, twin males, were fed normal colostrum from their dam for 72 hours. Following the colostrum feeding period all calves received, as an exclusive diet, photolyzed whole milk from the Ohio State University herd at the rate of 10 per cent of their body weight. The milk intake was reduced during periods of scouring. Calf *R* received, in addition to the milk, an average of 2.99 mg. of crystalline riboflavin per day. The calves were fed twice daily from nipple pails. The milk was heated to 37° C. before feeding, and the volume of milk fed was recorded. The calves were housed in a steam-heated calf barn and weighed at weekly intervals.

Riboflavin estimations were made on samples of the milk from each feeding, using a modification of the fluorimetric technique reported by Hand (3). Blood plasma vitamin A determinations were made periodically using the procedure of

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¹ Stored in frozen food containers generously provided by the Lily Tulip Cup Corporation, New York, N. Y.

Kimble (6). The method of Boyer *et al.* (1) was followed in estimating the vitamin A content of the milk and colostrum.

Approximately once each week, the experimental calves were placed on a 2 × 6-foot metabolism cage with a wire mesh floor and a 24-hour urine sample collected. A rubber feces-sack was attached to the calf to prevent contaminating the urine with feces. The urine was collected in a brown-glass bottle containing approximately 20 ml. of concentrated HCl. The final pH of the urine usually was about 1.5. The total volume was measured, and a representative sample was adjusted to pH 1.0, frozen and stored until assayed for riboflavin. The storage time averaged about 1 week, but in one case was as long as 6 weeks. Riboflavin determinations were made on the stored urine, using the fluorimetric technique of Slater and Morell (12). Recent work by these authors (11) has shown that human urine stored at a low temperature and a low pH gave lower riboflavin values upon analysis than when stored at a higher pH and at room temperature. No major discrepancies were observed in our results, however.

Since sunlight could not be depended upon as a constant source of photolyzing

TABLE 1

The effects of the photolysis treatment on the vitamin A, carotene and riboflavin of milk

Milk sample no. and treatment	Vitamin A		Carotene		Riboflavin	
	γ /liter	% loss	γ /liter	% loss	mg./liter	% loss
1. None	264		289		1.42	
1. Photolyzed	46	82.5	252	12.8	0.05	96.5
2. None	203		321		1.54	
2. Photolyzed	49	75.7	287	10.6	0.06	96.1

energy, a DH-1 400 watt mercury vapor lamp² was secured. This lamp emitted rays longer than 3000 Å, thereby eliminating excessive irradiation at the wave length of vitamin D activation. The lamp was used in conjunction with a highly polished parabolic aluminum reflector, and the rays were directed at a 2-gallon rectangular museum jar containing the milk. The milk was agitated slowly by continuous stirring. About 96 per cent of the riboflavin was destroyed by a 3-hour exposure. During the photolyzing period, the milk usually was allowed to reach a temperature of at least 60° C. for one-half hour as a bacteria-control measure.

The photolyzed milk was chalky white in appearance and had a pronounced "sunlight flavor". The milk seldom was over 36 hours old when fed and in all cases was kept under continuous refrigeration except during the period of light treatment. Vitamin A and carotene, as well as riboflavin, were destroyed in appreciable quantities, as shown in table 1. It will be noted that a treatment which destroyed over 96 per cent of the riboflavin also reduced the vitamin A by 75 to 80 per cent and the carotene by 10 to 12 per cent of their original quantities.

The biological inadequacy of the photolyzed milk was verified by a rat growth

² Purchased from the Westinghouse Electric Corporation.

test. Twenty weanling albino rats were divided equally into four groups as to size and litter. An attempt was made to distribute them equally as to sex, but one group (group 4) had three males and two females while all other groups had three females and two males. The groups were fed ad libitum the diets, as shown

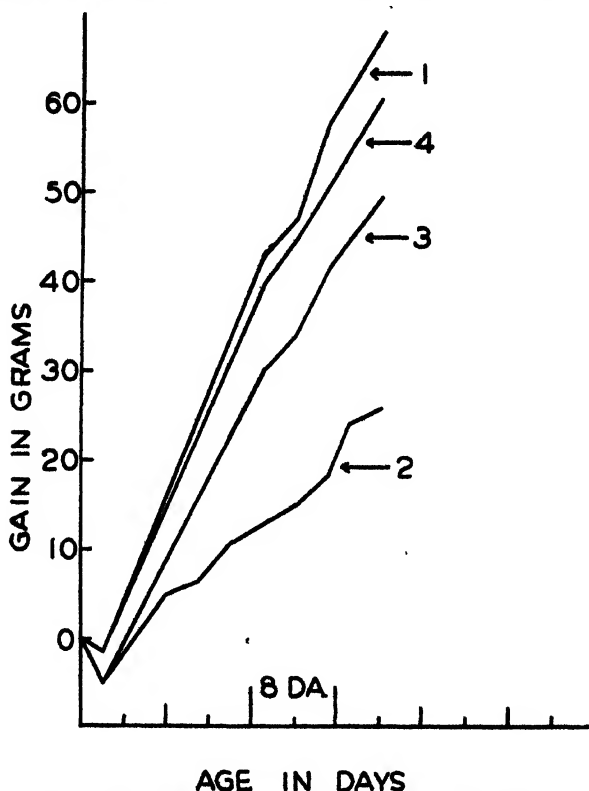


FIG. 1 Growth curves of rats fed the various mineralized milk diets:

Group	Diet	Grams gain/100 ml. consumed
1	Normal milk	6.10
2	Photolyzed milk	3.91
3	Photolyzed milk + riboflavin	5.22
4	Photolyzed milk + riboflavin + cod liver oil	6.06

One drop of a mineral mixture solution containing 500 mg. each of ferric citrate, manganous sulphate and copper sulphate in 500 ml. was added to each feeding.

Riboflavin in sufficient quantity to approximate that of normal milk was added to the milk of groups 3 and 4.

in the legend of figure 1. The respective rates of growth are recorded graphically (fig. 1). The gain per 100 ml. of milk consumed also is shown. Obviously the photolyzed milk had a low biological value for growth, and when such milk was

supplemented with both riboflavin and vitamin A the response was similar to that obtained from normal milk. The amount of gain per 100 ml. of milk consumed was almost identical (6.10 g. and 6.06 g., respectively, for Groups 1 and 4) indicating that the difference in the rates of growth was not due to a difference in nutritive value of the milk consumed but to a difference in total consumption. As the photolyzed milk had a pronounced "sunlight flavor", it is probable that the palatability of the photolyzed milk was lowered, and this in turn reduced the consumption.

Because of the low vitamin A content of the photolyzed milk, supplemental vitamin A was fed to each calf. The vitamin A oil was mixed with soya-lecithin

TABLE 2
Average daily riboflavin intake in milligrams

Age	Riboflavin intake values for Calf						
	A	B	C	R			
				In photo- lyzed milk	Ribo- flavin added	Total intake	If fed normal milk ^c
	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)
1 day	1.24	2.05	fb	fb		fb	
2 days	0.82	0.71	fb	fb		fb	
3 "	0.64	0.29	fb	fb		fb	
4-7 days	0.23	0.18	0.15	0.15	2.63	2.78	3.69
2 wks.	0.22	0.13	0.11	0.10	2.46	2.56	3.45
3 "	0.21	0.07	0.13	0.13	2.71	2.84	3.80
4 "	0.23	0.13	0.14	0.14	2.59	2.73	3.63
5 "	0.15	0.11	0.12	0.16	2.90	3.06	4.06
6 "	0.15	0.10 ^a	0.17	0.18	3.01	3.19	4.22
7 "	0.17	0.15 ^a	0.19	0.22	3.37	3.59	4.72
8 "	0.17	0.17 ^a	0.15	0.17	3.61	3.78	5.05
9 "	0.12	0.20	0.10	0.18	3.47	3.65	4.86
10 "	0.22	0.23					
11 "	0.15	0.19					
12 "		0.16					
Average, 4th day to term- ination	0.18	0.15	0.14	0.16	2.99	3.13	4.16

^a Calf B was fed 2 mg./day in addition to that present in the milk.

^b Calves were fed normal colostrum from their dams. No record was kept of riboflavin intake.

^c Calculated on the basis of the calf's intake of normal milk containing 1.4 mg. of riboflavin per liter.

(2) and a small amount of photolyzed milk in a Waring Blender. The amount of vitamin A fed varied from 5,000 to 25,000 I.U. daily.

The average daily riboflavin intake for each calf is recorded in table 2. During the colostrum feeding period, calves A and B received 2.7 and 3.0 mg. of riboflavin, respectively. This is less than 11 per cent of the amount found in an equal quantity of average Guernsey colostrum (16). Since calves A and B were not assigned to the experiment until 3 days of age, their riboflavin intake during the colostrum feeding period is unknown. From the fourth day to the termination of the experiment the daily riboflavin intake averaged about 4 per

cent of a normal intake with the exception of calf *R*. Assuming that normal milk contains about 1.4 mg./liter (an average found in our laboratory), this latter calf received approximately 72 per cent of the amount normally found in the milk prior to photolysis.

The growth rate of the calves is shown graphically in figure 2, with the Ragsdale standard (8) included for comparison. The growth of calf *A* was normal and uneventful for the first 4 weeks, except for a brief period of scours during the first week which responded to sulfathaladine medication. From the fourth week, the calf suffered from intermittent scours which showed little improvement on sulfathaladine treatment. The quantity of milk fed was reduced at each

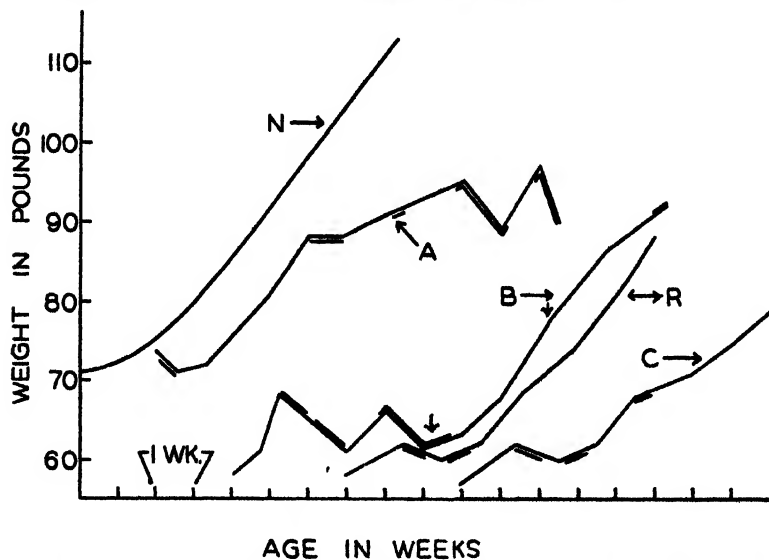


FIG. 2 Growth curves of calves used in this experiment. Double line indicates period of diarrhea. (N = Ragsdale standard, other letters refer to calves as designated in the text).

period of scours, and the calf never was able to consume the full allotment without scouring from the fourth week. An increasingly unthrifty condition developed, and obvious symptoms of riboflavin deficiency appeared at about 6 weeks. There were periods of excessive salivation and lacrimation. The haircoat became rough; there was evidence of a mild dry scaly dermatitis and a generalized alopecia which was more pronounced about the eyes and muzzle. A blood plasma ascorbic acid determination at 10 weeks showed 0.42 mg. per 100 ml., which was considered to be within the normal range. Periodic blood plasma vitamin A determinations (see table 3) showed normal levels, with the possible exception of the last two, which were taken towards the end of the experiment. These low values were considered to result from, rather than to be the cause of, the poor physical condition. Riboflavin excretions are shown in table 4. It will be noted that they remained low throughout the experiment.

TABLE 3
Blood plasma vitamin A of the calves used in the experiment

Age	Blood plasma vitamin A values for Calf:				
	A	B	C	R	Normal ^c
	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)
Birth	...	0.41		3.3
2 day	4.86				
3 "		15.86	11.43	13.36	14.7
4 "	4.99				
1 wk.	19.30				13.1
2 "		13.40			12.3
3 "	12.77	9.87	11.78	12.16	10.1
4 "					9.0
5 "	12.45		10.75	10.75	
6 "	10.90		8.90	8.99	
7 "		12.60	8.79 ^a	12.15 ^a	
8 "			9.49	13.45	9.7 ^d
9 "		13.8	6.33 ^b		
10 "	8.88	15.0			
11 "	9.96	12.1			
12 "					12.9 ^d

^a Vitamin A supplement was increased from 5,000 I.U. per day to 10,000 I.U. per day.

^b Level determined 3 days after preceding determination while animal was in a state of collapse.

^c Based on values obtained in this laboratory from nine Guernsey calves.

^d Based on average values from seven calves.

Calf A was sacrificed at 10.5 weeks of age and a gross post mortem examination showed marked catarrhal enteritis, mild edema of the cerebrum and "white spotted" kidney. One cornea was pebbled in appearance similar to that reported by Street et al. in the riboflavin deficient dog (15). The rumen contained approximately 1 liter of macerated straw and fluid. The rumen fluid contained 0.22 mg. per liter of riboflavin, which is about three times the concentration found in the milk consumed. Incubation of a portion of the rumen contents for 16 hours

TABLE 4
Urinary excretion of riboflavin by the calves used in the experiment

Age	Urinary riboflavin excretion by Calf:			
	A	B	C	R
	(mg./day)	(mg./day)	(mg./day)	(mg./day)
24 hr.	0.30			
36 hr.		0.10		
1 wk.	0.05		0.86	0.76
2 wk.	0.01	0.01		
3 wk.	0.01			
4 wk.	0.07		0.02	0.44
5 wk.		0.01		
6 wk.	0.03		0.05	0.60
7 wk.		0.05 ^a	0.05	0.38
8 wk.	0.03	0.09 ^a		0.64
9 wk.		0.04		
10 wk.		0.03		
11 wk.	0.03			

^a Received 2 mg. of crystalline riboflavin added to its diet during this period.

at 37° C. resulted in a 33 per cent increase in riboflavin concentration. These findings are considered as evidence of some microbiological synthesis, although insufficient in amount to fully protect the calf.

Calf *B* grew normally (fig. 2) for 1.5 weeks. Intermittent scours then resulted in poor growth until it was 5.5 weeks of age. At this time, the calf had been scouring for 9 days and was extremely unthrifty. It had a rough haircoat, a scaly dermatitis and mild alopecia which was more pronounced around the head. When a reduction in milk together with sulfathaladine treatment failed to improve the diarrhea, 2 mg. of crystalline riboflavin per day were added to the photolyzed milk for a period of 3 weeks. This addition to the diet resulted in the cessation of scours within 3 days, marked improvement in general appearance including the growth of new hair and the cessation of the excessive salivation and lacrimation. The amount of riboflavin excreted increased as shown in table 4. Growth was resumed almost immediately, and for the period of supplementary feeding it compares favorably with the Ragsdale standard. The

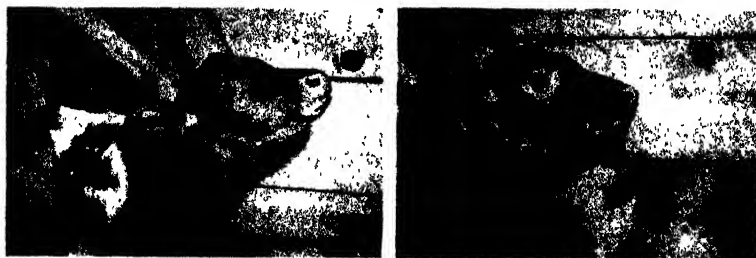


FIG. 3 Left, Calf *B* at 35 days of age, 3 days before 2 mg. of riboflavin were added to its daily diet. Note the excessive salivation. Right, The same calf at the conclusion of the 3 week supplement feeding period.

improvement of the deficiency symptoms following riboflavin therapy is shown in figure 3. Blood plasma vitamin A was within the normal range throughout the experiment, as shown in table 3. The blood plasma ascorbic acid at 9 weeks of age was 0.44 mg. per 100 ml. and was considered to be normal. Post mortem examination showed mild catarrhal enteritis as the only gross pathology.

The twin calves (*C* and *R*) differed by 1 lb. in weight at birth and critical body measurements were identical. They responded in an identical manner for the first 4.5 weeks, (fig. 1). At this point, the growth rate of calf *C* became slower while calf *R*, which was receiving a continuous daily riboflavin supplement, grew at a rate which approached the Ragsdale standard. No difference between these calves could be detected until after 4.5 weeks of age. From this time on, calf *C* was distinctly more lethargic, showed increased salivation and lacrimation, a dry scaly dermatitis and alopecia, particularly around the eyes and base of the ears. Calf *R* appeared completely normal.

About 2 weeks before calf *C* was sacrificed, it had marked difficulty in swallowing. It had to release the nipple several times during each feeding to clear its throat. About 48 hours prior to exsanguination it refused its feed. Thirty

hours later it was found in a state of acute collapse. It could not walk but was able to stand for a few seconds. A thin, watery salivation was excessive. The calf failed to respond to an intravenous injection of 500 ml. of "Intragel"³. Five mg. of riboflavin in physiological saline were injected intravenously 5 hours later. The calf was still alive 12 hours later but not noticeably improved in condition. At this point, the calf was destroyed and tissues obtained for microscopic study. The gross pathology consisted of marked catarrhal enteritis, a mild edema of the lungs and a few scattered spots on the kidney. No evidence of pneumonia was observed.

No gross pathology was found in calf *R* following exsanguination approximately 3 weeks later.

At 6 weeks of age the vitamin A supplement of both calves *C* and *R* was increased from 5,000 to 10,000 I.U. per day. It will be noted from table 3 that calf *C* failed to respond. This is further evidence that the low blood plasma vitamin A resulted from the riboflavin deficiency syndrome, probably the severe enteritis. Blood plasma ascorbic acid was within the normal range in both calves at 6 weeks of age (0.45–0.46 mg./100 ml.). Calf *R* gained 19.5 lb. per 100 l. of milk consumed, while calf *C* gained only 15.7 lb. on a similar amount. Thus, the difference in rate of growth can not be attributed entirely to the difference in the amount of milk consumed.

The residual effect of the high riboflavin intake during the colostrum feeding period is shown by a relatively high excretion in both calves at 1 week of age. Following this, the excretion in calf *C* decreased to a low level (see table 4) while that of calf *R* was maintained at a level 7 to 12 times higher for the remainder of the experiment. A single 24-hour urine collection from a 6 weeks old Guernsey calf which had been fed milk, hay and grain in the usual manner showed an excretion of 2.86 mg. per day. No clearent lesions of the lips, gums or eyes, with the exception of one pebbled cornea, were observed in any of the calves in this experiment.

The process of photolysis no doubt destroyed other vitamins, particularly ascorbic acid and pyridoxine. The partial or complete destruction of these was not considered as a complicating factor in these experiments for the following reasons: It has been shown that calves do not require a dietary source of ascorbic acid (17). A marked poikilocytosis which responded to pyrodoxine treatment has been described in adult cattle (9, 10). A few poikilocytes were found in the blood of only one calf in this experiment (calf *B*), and the number was no larger than one might expect to find in the mild anemia from milk feeding.

An approximate riboflavin requirement for a very young calf was determined from the data presented. Calf *R* made normal response to a diet of 3.13 mg. per day and grew from a weight of 58 lb. to 89 lb. or a mean of approximately 70 lb. Therefore, this calf responded normally to an average daily intake of 94 γ per kg. of body weight. Calf *B* responded at 5.5 weeks of age to an average daily

³ (8 g. of pyrogen-free gelatin per 100 ml. of 0.85 per cent saline) manufactured by Fort Dodge Laboratories Inc., Fort Dodge, Ia.

riboflavin intake of 2.15 mg. or, based on the weight of the calf (61 lb.), 77.4 γ per kg. of body weight. Since in vitamin deficiency diseases the amount required to cure the disease is usually larger than the amount required as a preventive, it seems logical that the growth requirement is even less than 77.4 γ per kg. of body weight. If calves were fed normal milk containing 1.4 mg. per liter at the rate of 10 per cent of the body weight, they would receive 140 γ per kg. which is almost twice the amount that was necessary to cure the symptoms of calf *B*. Thus, it seems that the possibility of a riboflavin deficiency developing during the milk feeding period is slight.

SUMMARY AND CONCLUSION

Approximately 96 per cent of the riboflavin in milk fed was destroyed by exposure to the radiations of a 400 W. mercury vapor lamp emitting light of wave lengths longer than 3,000 Å. Appreciable quantities of vitamin A and carotene also were destroyed. Four male Guernsey calves were fed this treated milk supplemented with adequate vitamin A. One of these calves also received approximately 2.99 mg. of added riboflavin daily.

Riboflavin deficiency symptoms consisted of erratic growth, intermittent diarrhea, a dry scaly dermatitis, alopecia, particularly about the head, periodic excessive salivation and lacrimation and in the acute stages, dysphagia and a peculiar collapse syndrome (one calf). Post mortem examinations showed evidence of catarrhal enteritis, mild edema of the lungs, (the collapse victim), pebbled cornea (one calf), mild edema of the cerebrum (one calf) and abnormalities of the kidney in two cases. These calves were extremely unthrifty. The addition of 2 mg. of riboflavin daily to the diet of one of these calves resulted in a prompt cessation of diarrhea, resumption of growth and a marked improvement in general appearance, including the growth of new hair. No other lesions of the lips or mouth or abnormalities of the eyes were noted.

The performance of the calf receiving 2.99 mg. of added riboflavin from the start was uneventful and approached the Ragsdale standard of growth.

Blood vitamin A and ascorbic acid levels were normal in all four calves. The urinary excretion of riboflavin varied from 0.01 to 0.06 mg. per day for calves receiving the treated milk with no added riboflavin and 0.38 to 0.64 mg. per day for the calf which received added riboflavin throughout the experiment.

The limited data of this experiment indicate that the minimum daily riboflavin requirement of the very young calf is somewhat less than 75 γ per kg. of body weight. The possibility of riboflavin deficiency during the milk feeding period is remote.

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SOME CHANGES IN DRY WHOLE MILK DURING STORAGE¹

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Numerous changes other than the oxidation of the lipides are known to occur in dry whole milk during storage. Those in flavor are most apparent, although changes which can be measured more objectively may be important and would be most valuable as indices of deterioration if they could be shown to be associated with flavor.

The general factors influencing the development of a tallowy flavor in dry whole milk now are well known. Actual tallowiness does not develop unless more than 0.3 ml. of oxygen per gram of powder is available, although Lea *et al.* (15) and Coulter (4) have shown that lower oxygen levels improve the keeping quality. Flavor deterioration is most rapid during the initial period of storage and once the oxygen has been exhausted from the free-space gas, low-moisture powder becomes almost stable in quality at normal storage temperatures (4, 15).

Stale and allied flavors in dry whole milk have never been exactly defined. Supplee (20), Tillmans and Strohecker (22), Hunziker (12) and many others in the industry have recognized stale, musty or gluey flavors believed to be associated with the protein fraction of the milk and have presented evidence that these flavors become more marked in high moisture powders. Lea *et al.* (15) noted the appearance, in powder stored at 47 and 37° C. for some time, of a flavor variously described as "heated," "burnt," "scorched" or "cooked". This flavor was considered to consist of two components, (a) a "burnt" or "caramel" taste associated with the protein or carbohydrate, and (b) a "butter-toffee" flavor associated with the fat.

Loss of solubility and browning accompany staling (12, 15). Doob *et al.* (8) have published extensive data on the influence of moisture on the browning of dried whey and skim milk. Tarassuk and Jack (21) have reported on the browning reaction in whole milk powder and ice cream powder since this investigation was completed. McCreary (16) has noted a decrease in soluble lactose in dried milk stored for a year at room temperature.

Lea *et al.* (15) noted the disappearance of oxygen in sealed cans of both skim and whole milk powders and the production of carbon dioxide.

Chapman and McFarlane (3) developed a method for determining acid ferri-cyanide reducing substances in dry milk and reported that they increased in dry whole milk stored in contact with the atmosphere. Modifications of the method and indications as to the source of the reducing materials have been presented by Lea (14) and Crowe *et al.* (6).

The production of fluorescent materials has been demonstrated to be asso-

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ciated with the deterioration of certain foods. Jenness and Coulter (13) have reviewed the literature on this subject and have suggested a method for evaluating the fluorescence characteristics of dry milk based on successive extractions with (a) 67 per cent acetone, (b) 20:80 acetone—ether and (c) 10 per cent KCl, and determinations of the blue fluorescence of each solution.

The browning reaction recently has been reviewed by Cantor *et al.* (2). Maillard, in the original publications concerning the reaction which now bears his name (Maillard or browning reaction), reported that the evolution of carbon dioxide, production of water (12 moles of water for every mole of carbon dioxide) and the formation of water-insoluble products accompanied the browning. Maillard also concluded that atmospheric oxidation played no part in the reaction.

Pearce (18) compared the suitability of a number of objective tests with subjective scores of palatability, and concluded that palatability was the most precise. This conclusion is not surprising considering the complexity of the system and the deficiencies in information concerning the deteriorative changes which occur.

The purpose of this research was to study changes which occur in dry whole milk during storage other than those associated with the lipides, and to secure some information on the effects of moisture, oxygen and temperature on these changes.

MATERIALS

Some observations were made using simplified systems as described by Jenness and Coulter (13). Briefly, these were prepared from acid-precipitated casein, dialyzed-milk serum protein, filtered milk fat, a concentrate of fat-globule "membrane" from washed cream and commercial samples of lactose and ascorbic acid and were dried from the frozen state.

Unless otherwise noted the experimental work to be reported on dry whole milk involved two large lots of powder, one (lot 384) of commercial origin and the other (lot K33) manufactured in an experimental spray drier in the University laboratories, using a standard procedure comparable to commercial practice. Portions of each lot were adjusted by exposure to humid air to secure samples varying in moisture content from 2.0 to about 5.0 per cent. The samples were canned and nitrogen-packed to secure oxygen levels ranging from approximately 1.0 to 6.0 per cent. Samples at each moisture and oxygen level were stored at 20, 37 and 60° C. The samples of the commercial powder stored at 60° C. were lost due to failure of the heat regulator of the incubator. The samples stored at 60° C. were analyzed at 10-day intervals up to 50 days, those at 37° C. at 4-week intervals up to 16 or 20 weeks and those at 20° C. at 8-week intervals up to 32 weeks. Some data were secured on other lots of dry whole milk manufactured in the experimental drier.

EXPERIMENTAL

Change in flavor on storage. Over 1,000 samples of dry whole milk representing both commercial and experimental production were scored by a selected

panel of five judges. A flavor described as "burnt feathers" was recognized in 84 samples. In only 9 instances was this criticism used in describing the flavor of dry whole milk containing less than 2.0 per cent moisture. Unless obscured by tallowiness or the caramelized flavor of powder which had become "brown," it was recognized in virtually all samples of stored powder containing more than 3.0 per cent moisture. Although it appeared more rapidly at the higher temperature, it was recognized in dry whole milk stored at 20, 37 and 60° C.

The flavors of the stored powders in lots 384 and K33 are typical. After 10 days at 60° C. the powder at the two highest moisture levels (3.81 and 4.49 initial) was brown and had the characteristic caramelized flavor. After 8 weeks at 37° C. and 16 weeks at 20° C., all samples, regardless of oxygen level, which contained more than 2.5 per cent moisture, were criticized as having the burnt feathers flavor. At the lower moisture levels, the samples were criticized as stale and finally tallowy at oxygen levels above 4.0 per cent.

In an attempt to determine the source of the burnt feathers flavor, frozen-dried simplified systems were prepared consisting of calcium phosphocaseinate with and without the addition of one or more of the following constituents: lactose, butterfat, serum protein, fat-globule membrane material and ascorbic acid. These were stored at 37° C. over sulfuric acid-water mixtures to obtain vapor pressures comparable with dry whole milk of low (2.5 per cent) and high (5.0 per cent) moisture. The calcium phosphocaseinate systems remained virtually unchanged in flavor. Those containing the phosphocaseinate and lactose, either with or without the addition of the other constituents, acquired the characteristic burnt feathers odor at the higher vapor pressure, and a characteristic stale flavor at the lower vapor pressure. The burnt feathers flavor therefore appears to be associated with lactose-protein changes and appears in high-moisture but not in low-moisture powder. The characteristic stale flavor that develops in normal dry whole milk probably is a composite of flavors resulting from lactose-protein changes and oxidation of the lipids. The flavor designated in this study as burnt feathers is probably the same as that described by others as "gluey."

Relation of moisture content to flavor deterioration. The importance of moisture content to the overall deterioration of gas-packed dry whole milk is shown graphically in figure 1. The samples involved are those gas-packed at the two lowest oxygen levels (below 2.0 per cent) from lots 384 and K33. Although, as shown by Lea *et al.* (15) and Coulter (4), the rate of loss in score of adequately gas-packed dry whole milk is not a straight line function of time but decreases with time, the total loss in score at any time interval can be used as an index of the overall rate of deterioration. The average weekly loss in score was computed from the difference between the original (fresh) score and the score after storage for the longest period of time, or in the case of the higher moisture samples at the higher storage temperatures, from the difference between original score and that at the last period at which an actual score was given. The logarithms of the weekly loss in score at 20, 37, and 60° C. with

one lot and at 20 and 37° C. with the other lot are shown in figure 1 plotted against the moisture content. The rate of loss in score appears to increase logarithmically with increase in the moisture content. Considerable variability in the rates of loss in score between the different lots of powder at any given moisture content and temperature is evident. It is realized that since the rate of loss in score of dry whole milk is not a straight line function of time, the comparisons made are empirical; however, the conclusions are believed to be

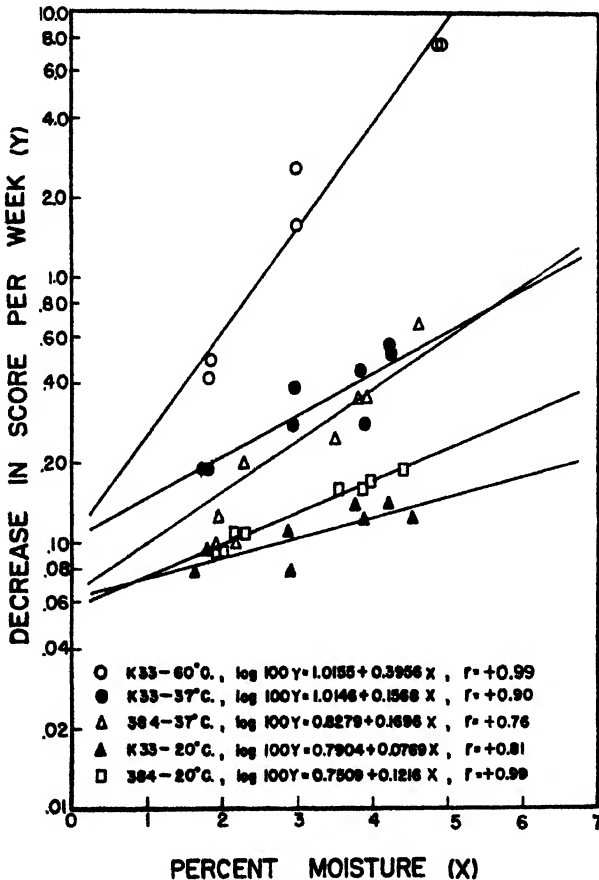


FIG. 1. The relationship of moisture content of dry whole milk to rate of loss in flavor score.

valid when dealing with adequately gas-packed dry whole milk. Holm and Greenbank (11) have shown that the minimum moisture content is not optimal in preventing tallowness of air-packed powder. Gyorgy *et al.* (9) and Williamson (23) have presented evidence indicating that certain antioxidants require moisture for effectiveness.

Acid ferriocyanide reduction. The total and non-protein acid ferriocyanide

reducing capacity was determined using the method described by Crowe *et al.* (6). Initial trials were made using 9 lots (162 samples) of spray-dried whole milk produced on the experimental drier. The moisture content of the lots was varied within the range of 1.92 to 4.78 per cent. In some instances moisture variation was secured by drying to different levels, in others by exposing portions of the powder in thin layers to a humid atmosphere. Part of the powder in each lot was packed in air in no. 2 cans, and part was gas-packed in

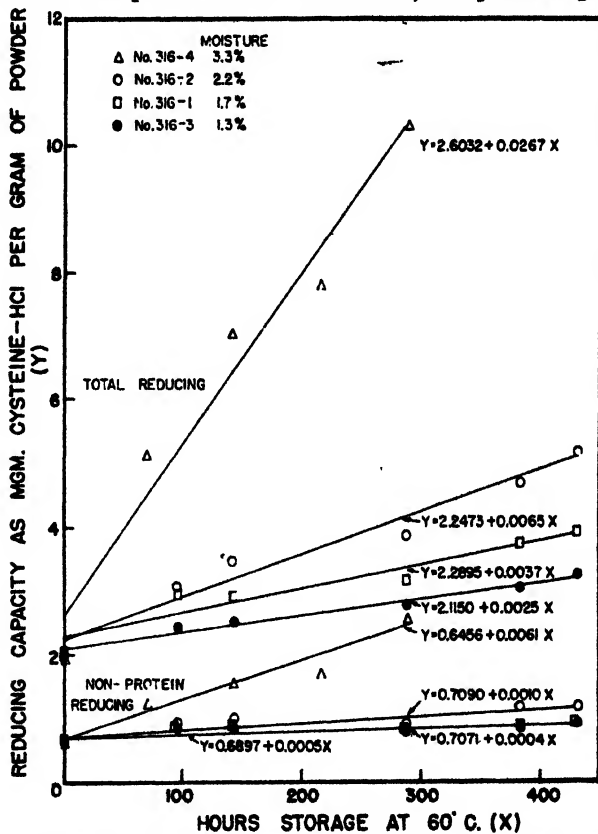


FIG. 2. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

nitrogen to secure an oxygen level of less than 2 per cent. The powder was stored at $60 \pm 1^\circ$ C. and samples for analysis removed at intervals up to 432 hours.

The data for one group of samples which are graphed in figure 2 show an increase in both total acid ferricyanide reducing substances and non-protein acid ferricyanide reducing substances with time of storage at 60° C. Since the relationship appears to be essentially linear, the regression line for each powder was computed. The rate of production of acid ferricyanide reducing sub-

stances increases with increase in the moisture content of the powder. The data for the other samples (not presented) indicate a similar relationship.

To show in a more striking manner the relationship of the moisture content of the powder to the production of acid ferricyanide reducing substances, the log slopes of the regression lines were plotted against the average moisture contents of the samples (fig. 3). An increase in the moisture content of the

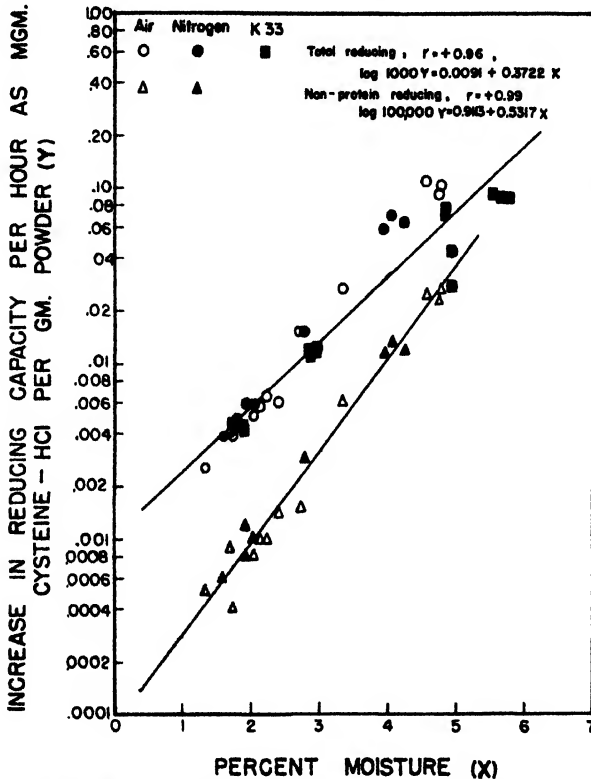


FIG. 3. The relationship of moisture content of dry whole milk to rate of increase in acid ferricyanide reducing capacity during storage at 60° C.

powder is accompanied by a logarithmic increase in the rates of production of both total and non-protein acid ferricyanide reducing substances. The data clearly indicate that the oxygen content of the atmosphere in contact with the samples is without effect on the rate of production of acid ferricyanide reducing substances.

The above observations were supplemented by data secured on lots 384 and K33. The results for the changes in the acid ferricyanide reducing capacity of the samples stored at 60° C. are plotted in figure 4. Since the oxygen level was without effect on the production of acid ferricyanide reducing substances, the

values shown for each moisture level are averages for the samples at the four oxygen levels.

The previously demonstrated linearity in the rate of production of acid ferricyanide reducing substances in dry whole milk stored at 60° C. is not maintained, particularly in the high moisture powders, on storage for longer than 10 days. In fact, a maximum may be reached followed by an actual reduction in the acid ferricyanide reducing capacity. This indicates a secondary reaction involving the non-oxidative utilization of the acid ferricyanide reducing substances.

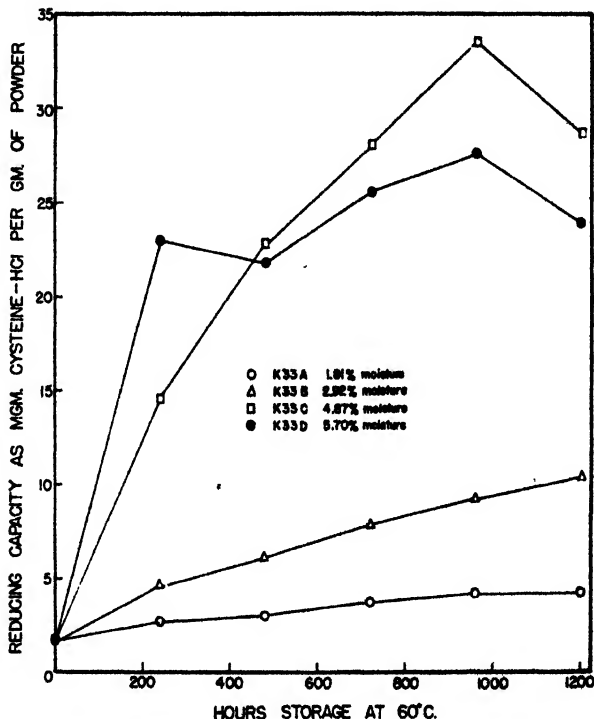


Fig. 4. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

The rate of production of acid ferricyanide reducing substances in each sample was computed from the initial and 10-day values. These data, plotted against moisture content in figure 3 confirm the relationship between the moisture content of the powder and the log of the rate of production of acid ferricyanide reducing substances during the initial storage period.

The data on the reducing capacity of the samples stored at 37 and 20° C. are summarized in tables 1 and 2. Since the oxygen level appears to have been without effect on the reducing capacity, only the average values for the samples at any given moisture level are shown. The initial reducing capacity of the

TABLE 1

Effect of storage at 37° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)					
	Weeks storage					
	Initial	4	8	12	16	20
Lot 384						
1.89	4.00	3.70	3.50	3.24	3.34	4.23
2.29	3.98	3.60	3.56	3.63	3.44	4.14
3.69	3.96	3.78	3.70	3.95	3.65	3.96
4.39	3.90	4.05	5.61	6.22	8.55	9.47
Lot K33						
1.75	1.61	1.96	1.88	1.74	1.86	
2.32	1.75	1.97	1.86	1.91	2.08	
3.81	1.58	2.05	2.07	2.28	2.38	
4.33	1.61	2.28	2.16	2.86	2.72	

commercial powder (lot 384) was somewhat greater than twice that of the powder made in the experimental drier (lot K33). This difference may be due in part to a greater reducing capacity of the fluid milk, but probably is due primarily to differences in heat treatment during processing [see Crowe *et al.* (6)]. There was a slight decrease in the acid ferricyanide reducing capacity of the samples stored at 20° C., and except for the powder of the highest moisture content (4.39%), there was a slight decrease in the acid ferricyanide reducing capacity of the commercial powder up to 16 weeks storage at 37° C. The values at 20 weeks were about equivalent to those for the fresh powder. The acid ferricyanide reducing capacity of the samples highest in moisture increased materially during storage. The reducing effect of the low-moisture experimental powder samples was virtually unchanged during storage for 16 weeks at 37° C., but there was a definite, although minor, increase in the higher moisture samples.

TABLE 2

Effect of storage at 20° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)				
	Weeks storage				
	Initial	8	16	24	32
Lot 384					
1.90	4.00	3.46	2.97	3.32	3.11
2.32	3.98	3.49	2.94	3.32	3.05
3.81	3.96	3.51	3.28	3.19	3.10
4.46	3.90	3.49	3.30	3.30	3.11
Lot K33					
1.75	1.61	1.61	1.56	1.49	1.45
2.32	1.75	1.61	1.64	1.47	1.47
3.81	1.58	1.72	1.55	1.49	1.49
4.41	1.61	1.71	1.77	1.53	1.56

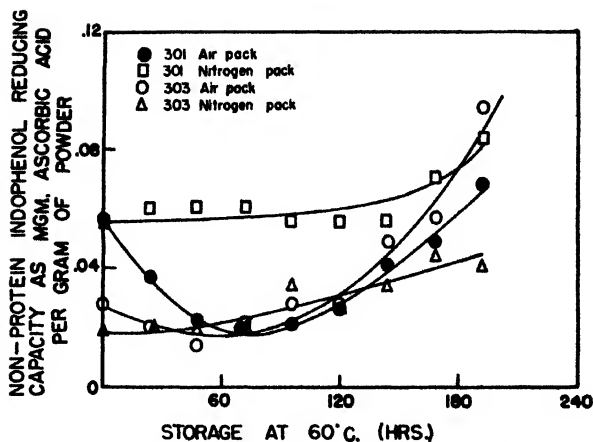


FIG. 5. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

These data demonstrate the marked effect of temperature on the rate of production of acid ferrieyanide reducing substances. At 20° C. the rate is so slow as to be negligible. At 37° C. the reaction is of minor importance except in powders of higher moisture content.

Ascorbic acid changes. The apparent ascorbic acid content of the samples

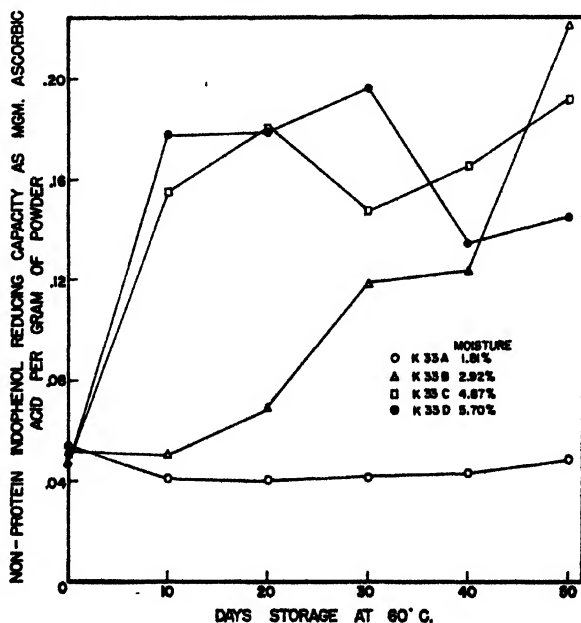


FIG. 6. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

was determined on the protein-free filtrates titrating with 2,6-dichloro-benzeneindophenol. The protein-free filtrates were secured by precipitation either with tungstic acid in the manner described by Crowe *et al.* (6), or with a mixture of trichloroacetic and metaphosphoric acids according to the procedure outlined by Doan and Josephson (7). In the presence of oxygen, the ascorbic acid is gradually oxidized in dry whole milk. As shown in figures 5 and 6, substances which react with indophenol are produced particularly in powders of higher moisture.

Fluorescence changes. Fluorescence was determined by the method of Jenness and Coulter (13). Since the oxygen content appeared to be without effect on fluorescence changes during storage, the results at each moisture level have been averaged without regard to oxygen level.

No change in fluorescence of either extract I or II resulted from storage at 20° C., but sample 384 showed a marked increase in fluorescence of extract III which was not duplicated by sample K33. The greater susceptibility of sample 384 to development of fluorescence was even more apparent on storage at 37° C. Sample K33 exhibited scarcely any increase in fluorescence of extract I and only a small increase in extract III during storage for 16 weeks at this temperature. In sample 384, on the other hand, marked increases in fluorescence of both of these extracts occurred. The fluorescence of extract II was unaffected in either sample. Figure 7 shows the changes in fluorescence of sample K33 during storage for 50 days at 60° C. Under this more drastic condition, the fluorescence of extract I increased sharply if the moisture content was sufficiently high, and some increase was noted in fluorescence of extract II. The effect on extract III is interesting in that at moisture contents in excess of about 4 per cent, the initial sharp rise was followed by a pronounced decrease in fluorescence, due either to destruction or insolubilization of the fluorescing materials.

In general, then, it appears that temperature and moisture level determine whether and to what extent fluorescing materials are produced during storage. A considerable difference between powders in susceptibility to production of these materials is also evident.

Production of carbon dioxide. The head-space gas of the cans was analyzed for carbon dioxide and oxygen with a Fisher Precision Gas Analyzer equipped with a Continental Can Company sampling device. All readings were computed to standard temperature and pressure in the manner described by Coulter and Jenness (5).

Carbon dioxide was produced at rates varying with the temperature and the moisture content. Typical data for the powder held at 60° C. are shown in figure 8. Since the rate of production of CO₂ appears to be essentially a straight line function of time over the period studied, the regression line for each powder was computed. The log slopes of the regression lines for the samples held at 60 and 37° C. are shown in figures 9 and 10, plotted against the moisture content. The 60° C. data include those for lot K33 and those for the samples in

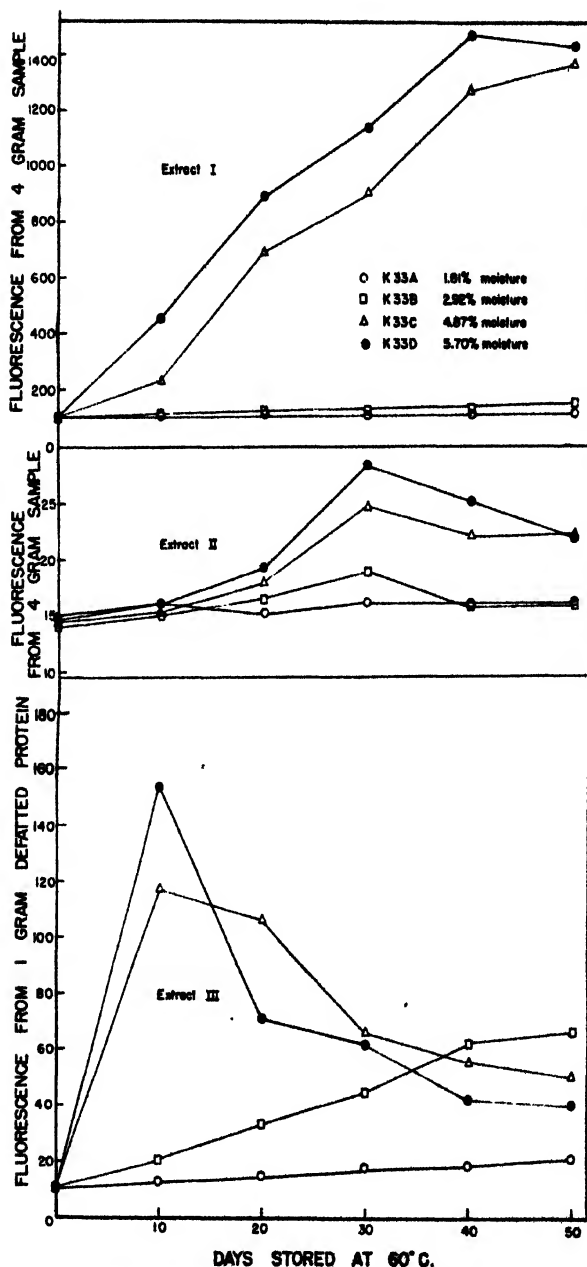


Fig. 7. Change in extractable fluorescence of dry whole milk stored at 60° C.

the initial 9 lots all of which were held for only 10 days. Although the data show considerable scatter, it is evident that production of CO_2 at any given temperature is an exponential function of the moisture content. The effect of oxygen level on the rate of production of CO_2 is not entirely clear. As shown in figure 9, the rate of production of CO_2 in the air-packed samples appears to be slightly higher than in the gas-packed samples. However this difference is far from uniform, and the effect of the oxygen level, if it is a factor, is minor in comparison to that of the moisture content. Production of CO_2 in the samples held at 20°C . was so slow that consistent rate data were not obtained.

Since the powder absorbs some of the carbon dioxide, Coulter and Jenness (5) and Pearce (19), the total carbon dioxide produced cannot be computed from the volume and analysis of the gas.

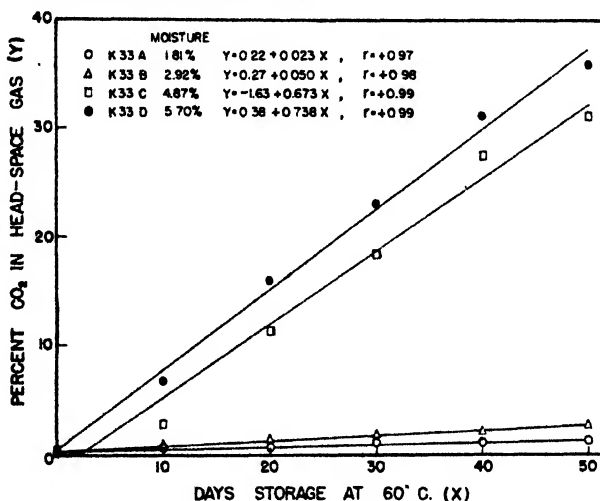


FIG. 8. Change in per cent CO_2 in head-space gas of dry whole milk stored at 60°C .

Oxygen utilization. Only a limited amount of data were secured which were adequate to establish rates of oxygen utilization. These were for the initial nine lots which were held at 60°C . for 10 days. The regression lines for oxygen utilization for each of the air-packed samples were computed and log slopes of the regression lines are shown plotted against the per cent moisture in figure 11. These data indicate that the rate of oxygen utilization increases logarithmically with increase in the moisture content. With these samples there was a very high correlation (+0.92) between the rate of oxygen utilization and carbon dioxide production. Since the rate of carbon dioxide production was only slightly greater in the air-packed than in the nitrogen packed samples, direct oxidation involving the free-space oxygen can play only a minor role in carbon dioxide production.

Production of water. The moisture content of the samples was determined at each examination period using the American Dry Milk Institute toluene dis-

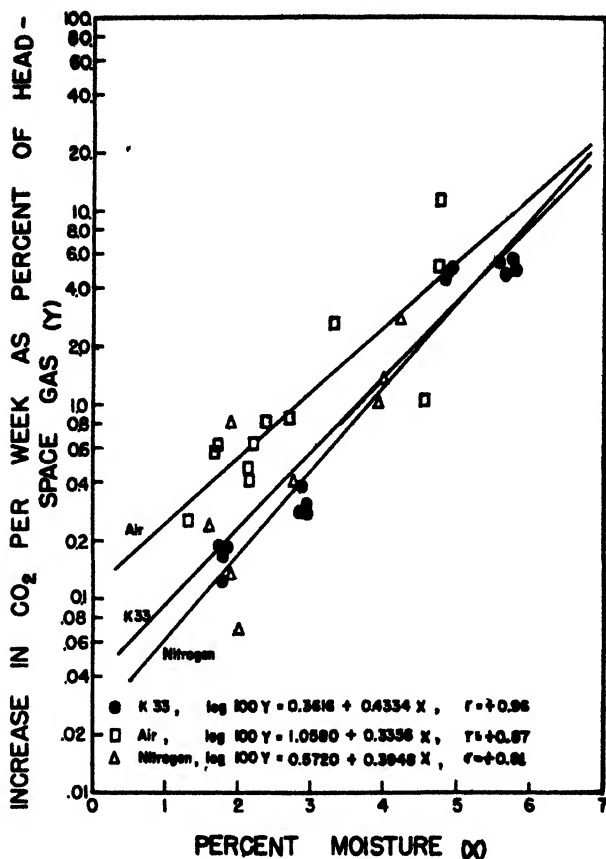


FIG. 9. Relationship of moisture content to rate of increase in CO_2 in head-space gas of dry whole milk stored at 60°C . in air and nitrogen. Sample K33 was stored at oxygen levels ranging from about 1 to 6 per cent.

tillation method. Since the oxygen content appears to be without effect on changes in the moisture content during storage, the results at each moisture

TABLE 3

Effect of storage at 60°C . on the moisture content of dry whole milk

Days of storage					
0	10	20	30	40	50
% moisture					
1.90	1.90	1.60	1.80	1.76	1.90
2.88	3.00	2.95	2.95	2.89	3.02
3.81	4.13	4.95	5.13	5.25	5.95
4.49	5.15	5.94	5.78	6.24	6.53

level have been averaged without regard to oxygen level. The data for the samples stored at 60° C. are shown in table 3. There was a definite increase in the moisture content of the samples having initial moisture levels of 3.81 and 4.49 per cent but not in lower moisture content samples. There was no change in the moisture content of any of the samples stored at 37 and 20° C.

Change in protein solubility. The soluble nitrogen of the samples was de-

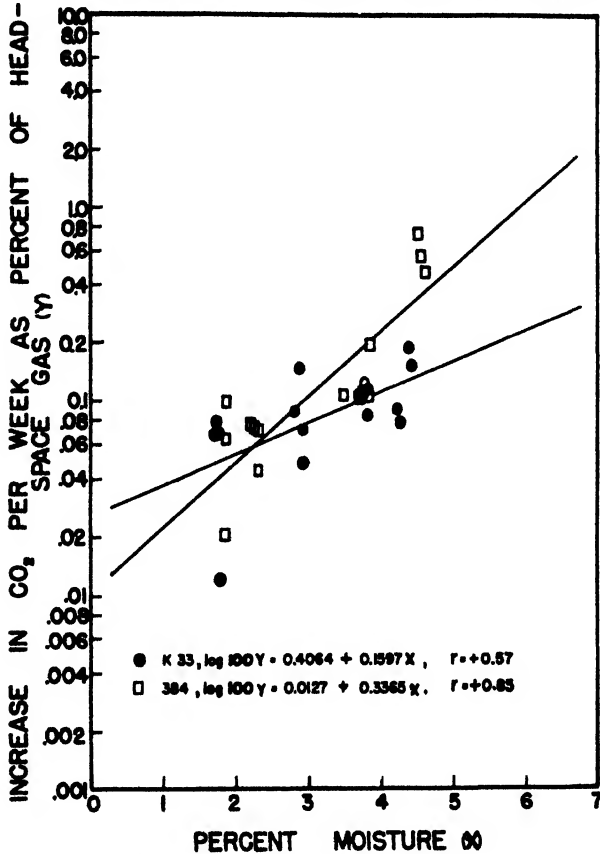


Fig. 10. Relationship of moisture content to rate of increase in CO₂ in head-space gas of dry whole milk stored at 37° C.

terminated on an aliquot taken from the center portion of the centrifuge tube following treatment of the milk according to the American Dry Milk Institute method for solubility index. This method was not entirely satisfactory for those samples which had become brown, since the treatment did not effect a sharp separation of the insoluble material. Filtration of the brown samples was found more satisfactory. The results for the sample stored at 60° C. are summarized in table 4. Since the oxygen level was without effect on protein

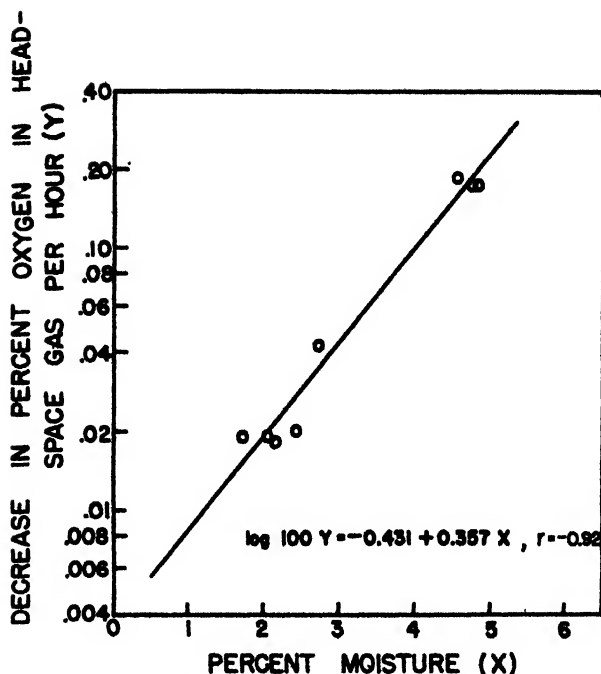


Fig. 11. Relationship of moisture content to rate of decrease in oxygen content of headspace gas of dry whole milk stored at 37° C.

insolubilization, the values reported are the averages at each moisture level. At the end of 50 days storage, the soluble nitrogen in the samples containing 4.49 per cent moisture had been reduced to 6 per cent of the original value. This means that virtually all of the protein was rendered insoluble by the experimental conditions. The solubility of the nitrogenous substances was not

TABLE 4

The effect of moisture level and storage at 60° C. on the lactose, soluble nitrogen and pH of dry whole milk

Storage period in days	% lactose ^a				Soluble N (Mg. per 100 ml.)				pH			
	Initial % Moisture											
	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49
0	36.30	36.65	36.05	36.03	503	502	505	508	6.47	6.49	6.49	6.41
10	36.05	34.30	33.67	33.27	506	495	50.2 ^b	70.3	6.49	6.46	6.17	5.87
20	35.30	33.30	33.17	32.40	504	482	52.7	39.7	6.47	6.32	5.63	5.64
30	35.20	33.23	32.57	31.57	502	458	38.6	33.0	6.45	6.48	5.43	5.38
40	34.85	33.06	32.20	31.37	489	452	36.4	30.3	6.42	6.40	5.32	4.97
50	35.75	33.55	32.56	31.57	495	468	29.5	30.4	6.48	6.32	5.10	4.99

^a Moisture-free basis.

^b The average of two filtered samples, the average of two centrifuged samples being 394.

affected by storage at 60° C. at the 1.90 per cent moisture level and only slightly affected at 2.87 per cent moisture. The solubility of the protein was unchanged in all of the samples stored at 37 and 20° C.

Change in lactose content. The lactose was determined by a modification of the chloramine-T method of Hinton and Macara (10), which is based on the stoichiometric oxidation of the aldehyde group of the sugar. Zinc hydroxide was used for the deproteinization of the milk as suggested by McDowell (17).

The per cent of lactose in the samples stored at 60° C. is shown in table 4. Since the oxygen level did not affect the lactose content, the values shown are the averages at each moisture level. Storage of dry whole milk with an initial moisture content of 4.49 per cent at 60° C. for 40 days resulted in a maximum loss of 15 per cent of the lactose as measured by this method. The lactose decreases most rapidly during the first 10 or 15 days of storage under these conditions. The tendency for an increase in the lactose content at 50 days over that at 40 days may be due to a reduction of the chloramine-T reagent by the increased concentration of competitive reducing systems in the browned milk. The decrease in reducing sugar at the 1.90 per cent moisture level was only 4 per cent.

The lactose content of the samples stored at 37 and 20° C. was unchanged.

Changes in pH. The pH of the reconstituted samples was determined using a Leeds and Northrup glass-electrode pH meter. The pH values for the samples stored at 60° C. are shown in table 4. The data reported are average values at each moisture content, as the oxygen level had no effect on the pH of the reconstituted milk. There was a marked decrease in the pH of the samples having an initial moisture content of 4.49 per cent; a lesser decrease at the 3.80 and 2.87 per cent moisture levels; but none at 1.90 per cent. The pH of the samples stored at 37 and 20° C. for 20 and 32 weeks, respectively, was unchanged.

DISCUSSION

Minimal oxygen levels are considered desirable for the storage of dry whole milk to prevent oxidative changes. Minimal moisture levels have not been considered optimal, because some moisture is necessary for the effectiveness of certain antioxidants. In adequately gas-packed powder, however, oxidation cannot occur.

Numerous changes take place in dry whole milk during storage which are accelerated by increase in the moisture level. These include development of stale, or, at higher moisture levels, a burnt feathers flavor, production of acid ferri cyanide reducing substances, production of carbon dioxide and utilization of oxygen. The rate of change of each of these, at least during the initial stages, has been shown to increase logarithmically with increase in moisture content. Other changes also increasing in rate with increase in moisture content but for which adequate rate data were not secured are: production of indophenol reducing substances, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss of

protein solubility. Thus, in adequately gas-packed dry whole milk, a minimal moisture content appears to be desirable; however, the rates of change in every instance are very slow in powders containing 2 per cent or less moisture.

Barker (1) in 1933 observed a logarithmic increase in the rate of heat denaturation of egg albumin with increase in the relative vapor pressure. He explained his observations on the basis that the relative humidity affected the freedom of the water molecules to move between and among the relatively immobile protein molecules and aggregates. He considered this interpretation pertinent regardless of whether the water was a reactant or merely a medium in which the reaction occurred. A similar relationship appears to hold for the reactions involved here.

Although vapor pressure determinations were not made on the samples involved in these trials, determinations on other samples covering the same range in moisture content showed that there is an essentially linear relationship between the per cent moisture and the relative vapor pressure.

All of the reactions mentioned are accelerated greatly by increase in temperature. The magnitudes of the various changes are unimportant except in samples which are higher in moisture than is considered desirable in commercial practice. Therefore, none of the objectively measurable changes can be considered as an effective index of deterioration in commercial dry whole milk.

SUMMARY AND CONCLUSIONS

The following non-lipid changes occur in dry whole milk during storage: development of a stale or burnt feathers flavor, production of acid ferrieyanide and indophenol reducing substances, production of carbon dioxide, utilization of oxygen, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss in protein solubility. All of these changes increase in rate with increase in moisture content and temperature but appear to be relatively unaffected by oxygen.

The rate of change in those variables for which adequate data were obtained increased logarithmically with increase in the vapor pressure of the water in the system, at least during the initial stages.

ACKNOWLEDGMENTS

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IN MEMORY OF ROBERT BEAR STOLTZ

On October 2, 1948, the bottom seemed to drop out of many, many lives, for on that day Robert Bear Stoltz was called to a greatly needed rest by his Maker. Great was the loss felt by his family and the host of friends he left behind. On the day of his burial, even the heavens wept unashamedly.

The greatness of Robert Stoltz can best be measured by the record of his achievements. His motto could easily have been, "What do we live for, if it is not to make life less difficult for others".

He was first of all a wonderful husband and father of four children, and took great pride and joy in his five grandchildren.

He was a true friend and wise councilor, none ever were better, for he had the wisdom, tact and diplomacy that rarely is found today.

He truly was a great educator, administrator and organizer.

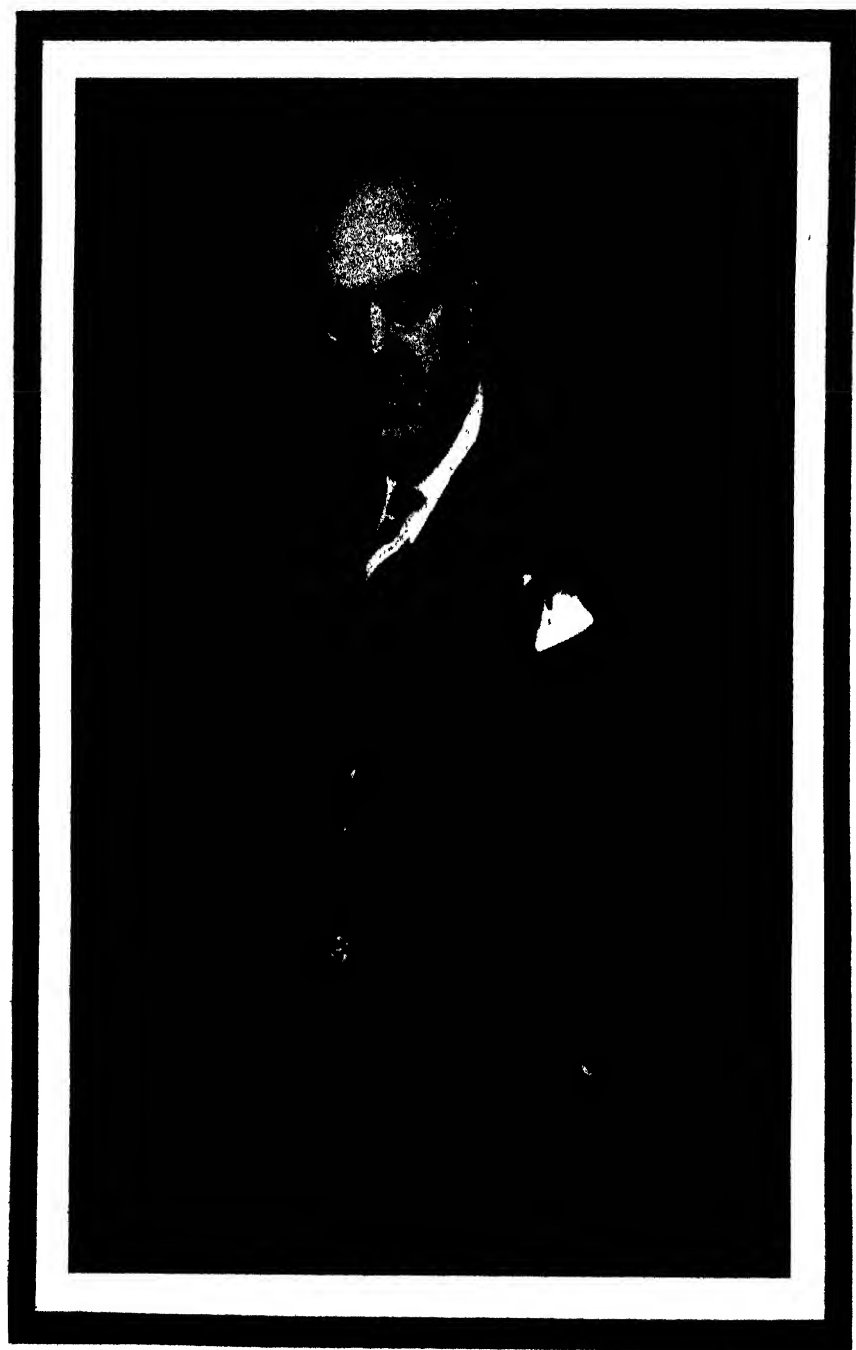
Professor Stoltz was 58 years old, a relatively young man, but one who had crammed more genuine greatness and achievement in that short span than many who live 20 to 30 years longer. He was an untiring and thorough worker.

Professor Stoltz had been associated with the Ohio State University, College of Agriculture since his graduation in 1912. In 1923 he was promoted to full professor, and in 1929 he was made the chairman of the then newly organized department of Dairy Technology. This department soon became outstanding under Professor Stoltz' guidance for the backing it received from the dairy industry in the State. He was instrumental in the organization of the Ohio Swiss Cheese Association in 1918, and was its secretary and treasurer until 3 years ago. He also promoted the organization of the Columbus Milk Distributors Association in 1934 and was its secretary until 3 years ago. This organization has done much to promote friendly relations among its members. He was also instrumental in their adoption of the universal milk bottle shortly after the organization was founded.

Other organizations recognized Professor Stoltz' ability. He was at one time secretary of the National Cheese Association, and since 1936 he was secretary-treasurer of the American Dairy Science Association, after being president of this organization in 1934. He did much to increase the membership of this organization and put it on a sound financial basis. In 1947 he was presented with the Association Award giving him honorary life membership in the ADSA. He was also a member of the Advisory Council of Sealtest, Inc.

In 1937 he made a study of dairying in New Zealand and Australia.

Ever mindful of both education and the dairy industry, he believed in turning out students well versed in the fundamentals of dairying and in the psychology of "how to win friends and influence people."



Because sufficient funds were unavailable at Ohio State University for research in dairy technology, Professor Stoltz, as an honorary member of the Board of Trustees of the Ohio Dairy Products Association in an advisory capacity, suggested and worked diligently toward the promotion of a research fund by the dairy industry in the State. This fund, known as the Ohio Dairy Products Research Fund, became a reality and today amounts to considerably more than \$100,000, the interest on which at 6 per cent is used for research in dairy technology at The Ohio State University. It has since been suggested that the name of this fund be changed to the Robert B. Stoltz Memorial Research Fund.

Professor Stoltz was listed in *Who's Who in America*, *Who's Who in American Education*, and in *American Men of Science*. He was a member of the Ohio Post-War Program Commission, and of the Columbus Rotary Club.

Professor Stoltz was very active in Masonry, and if ever a man was a true Mason and lived up to the Masonic Creed, it was Bob Stoltz. He was a 33rd degree Mason, and was elected this year as Deputy General Grand Master of the General Grand Council, R. & S. M., of the United States; had served as Grand Master of the Grand Council, R. & S. M. of Ohio; was Past Master of University Lodge and for 14 years its secretary; a member of York Chapter, R. A. M.; York Council, R. & S. M.; Columbus Commandery, Scottish Rite; Aladdin Temple; and Red Cross Constantine.

He was a member of Acacia Fraternity, and Delta Theta Sigma and Gamma Sigma Delta honorary fraternities.

Professor Stoltz was a native of Bradford, Ohio, where he was born March 6, 1890. Surviving are his widow, Mrs. Marie Cassel Stoltz, a son, Philip, three daughters, Mrs. Bonnie Marie Downes, Mrs. Susan Ann George, and Mrs. Roberta Mary Miles.

"Patience, kindness, generosity, humility, courtesy, unselfishness, good temper, guilelessness, sincerity—these make up the supreme gift, the stature of the perfect man." If that is so, then Bob Stoltz was a perfect man because he possessed all of them to a very marked degree.

L. H. BURGWALD

THE INFLUENCE OF WATER LEVEL AND TEMPERATURE OF STORAGE ON CAROTENE PRESERVATION IN DEHYDRATED ALFALFA, CEREAL GRASSES AND MIXED FEEDS¹

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In earlier papers (2, 3), it was pointed out that the carotene of dehydrated alfalfa or dehydrated cereal grasses could be preserved completely if the water content was adjusted to 12 to 20 per cent level and the material stored at 22 to 25° C. in airtight containers. It was postulated that this preservation was related to the speed of reaction of respiratory enzymes which in turn was related to the moisture content of the material.

EXPERIMENTAL

Further studies now have been made with lower water levels in these dehydrated materials stored for 3 months at 22 to 25° C. and 33 to 36° C., respectively. The water levels ranged from 0.9 to 15 per cent with graded increments generally of 2.5 per cent. The materials used were: (a) A dehydrated commercial alfalfa prepared in October, 1947, with an initial carotene content of approximately 350 γ per g. and a water content of 3.6 per cent. (b) An alfalfa cut from a University field in September, 1946, and dried in the laboratory at 50° C. This product was dried further in a vacuum oven for 24 hours at 50° C. prior to starting the experiment. The material, when put up for experimental observation, had a carotene content of approximately 150 γ per g. It consisted of both stem and leaf. (c) A dehydrated alfalfa² which was dried for 2.5 hours at 95° C. before being used in these water level experiments. It contained 154 γ of carotene per g. (d) A dehydrated cereal grass² which contained approximately 160 γ of carotene per g. and was dried for 2.5 hours at 95° C. before being used in the experiments.

All of these materials were adjusted to different water levels and placed in ice cream cartons holding about 250 g. The control was unwaxed, and the remaining cartons were dipped several times in Flexowax to insure complete exclusion of oxygen. They then were stored at 22 to 25° C., and duplicate sets stored at 33 to 36° C. for 3 months. At the end of that time, the cartons were opened, sampled for carotene determination and observations on color and aroma made. The data giving the results are found in tables 1 through 4.

Since it was possible to preserve the carotene in these dehydrated materials

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² Supplied by the Cerophyl Laboratories, Inc., Kansas City, Mo.

TABLE 1

*Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa a.
(Carotene data on water free basis)*

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	5.2	338	118	67.0
Sealed	Green	None	0.9	338	223	34.0
Sealed	Green	None	2.5	338	248	26.0
Sealed	Green	None	5.0	356	338	5.3
Sealed	Green	None	7.5	356	344	3.6
Sealed	Green	None	10.0	356	362	0.0
Sealed	Slight olive green	None	12.5	356	371	0.0
Sealed	Olive green	None	15.0	356	376	0.0
Stored at 33 to 36°						
No seal	Green	None	3.6	338	66	81.5
Sealed	Green	None	0.9	338	230	32.0
Sealed	Green	None	2.5	338	259	23.0
Sealed	Green	None	5.0	356	329	8.0
Sealed	Green	None	7.5	356	348	2.5
Sealed	Olive green	Positive	10.0	356	350	2.0
Sealed	Brown	Positive	12.5	356	363	0.0
Sealed	Brown	Positive	15.0	356	356	0.0

by the procedure outlined, it seemed important to study the losses of carotene in a mixed feed such as is often used in dairy, poultry and hog rations. The ration fed consisted of: soybean meal, 20 per cent; wheat middlings, 20 per cent; wheat bran, 10 per cent; white corn, 21 per cent; oats, 10 per cent; alfalfa

TABLE 2

*Effect of water level and temperature on carotene, color and pressure of air dried alfalfa b.
(Carotene data on water free basis)*

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	6.9	164	122	25.6
Sealed	Green	None	3.4	157.6	129	18.2
Sealed	Green	None	5.0	157.6	136	13.7
Sealed	Green	None	7.5	157.6	144	8.4
Sealed	Green	None	10.0	157.6	146	7.0
Sealed	Slight olive green	None	12.5	157.6	159	0.0
Sealed	Olive green	None	15.0	164	162	1.2
Stored at 33 to 36° C.						
No seal	Green	None	5.2	164	70	57.0
Sealed	Green	None	3.4	157.6	115	27.0
Sealed	Green	None	5.0	157.6	120	24.0
Sealed	Green	None	7.5	157.6	131	17.0
Sealed	Slight olive green	None	10.0	157.6	140	11.0
Sealed	Slight olive green	Positive	12.5	157.6	136	14.0
Sealed	Olive green	Positive	15.0	164	147	11.0

TABLE 3

Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa c. (Carotene data on moisture free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	4.4	154	95	38.0
Sealed	Green	None	3.7	154	111	28.0
Sealed	Green	None	5.0	154	117	24.0
Sealed	Green	None	7.5	154	138	10.4
Sealed	Green	None	10.0	154	139	9.7
Sealed	Slight olive green	None	12.5	154	149	3.2
Sealed	Slight olive green	None	15.0	154	159	0.0
Stored at 33 to 36° C.						
No seal	Green	None	3.1	154	68	55.0
Sealed	Green	None	3.7	154	104	32.0
Sealed	Green	None	5.0	154	116	24.0
Sealed	Green	None	7.5	154	132	14.0
Sealed	Slight olive green	None	10.0	154	144	6.5
Sealed	Olive green	None	12.5	154	154	0.0
Sealed	Olive green	Positive	15.0	154	159	0.0

meal, 15 per cent; CaCO_3 , 2 per cent; $\text{Ca}_3(\text{PO}_4)_2$, 1 per cent; and iodized salt, 1 per cent. The only major source of carotene in this feed was the 15 per cent alfalfa meal. The feed was stored at 33 to 36° C. for 3 months with water levels

TABLE 4

Effect of water level and temperature on carotene, color and pressure of Dehydrated cereal grass d. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	4.4	166	110	34.0
Sealed	Green	None	1.6	166	115	31.0
Sealed	Green	None	2.5	166	123	26.0
Sealed	Green	None	5.0	166	133	20.0
Sealed	Green	None	7.5	166	146	12.0
Sealed	Green	None	10.0	166	155	6.3
Sealed	Faint olive green	None	12.5	166	161	3.0
Sealed	Olive green	None	15.0	166	174	0.0
Stored at 33 to 36° C.						
No seal	Green	None	3.0	166	74	55.0
Sealed	Green	None	1.6	166	104	37.0
Sealed	Green	None	2.5	166	112	33.0
Sealed	Green	None	5.0	166	136	18.0
Sealed	Faint olive green	None	7.5	166	150	9.7
Sealed	Olive green	None	10.0	166	164	1.2
Sealed	Olive green	None	12.5	166	164	1.2
Sealed	Brown	None	15.0	166	172	0.0

ranging from 2.3 to 15 per cent under sealed and unsealed conditions. The initial moisture was reduced by drying for 2.5 hours at 95° C. A companion series also was set up but with the addition of certain trace elements now commonly used in such mixed feeds. This series which contained the trace elements was dried initially for 32 hours at 50° C. in a vacuum oven. The trace elements used were 0.02 per cent $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.02 per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.25 per cent $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 0.02 per cent $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$.

At the end of 3 months, these cartons were opened, carotene determinations made, as well as observations on color, flavor, aroma, rancidity and other characteristics. The initial carotene content of this feed was 39.6 γ per g. It is assumed that practically all of the carotene was in the 15 per cent of alfalfa meal.

The determination of carotene in the mixed feed by the Wilkes method (4) gave high results. This method seemed entirely satisfactory where only alfalfa or a cereal grass was involved, but when applied to the mixed feed with 15 per cent of alfalfa, the results often were 50 per cent too high. This assumes that there was complete carotene conservation with 12.5 to 15 per cent of water when sealed. Apparently, certain interfering pigments which developed during storage were registering as carotene by the Wilkes method.

The Wilkes method was abandoned for the mixed feed analysis, and the general procedures for chromatographic analysis of carotene which are given in *Methods of Vitamin Assay* (1) were incorporated in the determination. The extraction consisted of allowing 1 to 2 g. of sample to stand 16 to 18 hours (in the dark) in 60 ml. of a 2:1 mixture of Skelly B and acetone. After filtering to remove the sample, the extract was evaporated on a steam bath to reduce the total volume to about 50 ml. Saponification was accomplished next by adding 80 ml. of 5 per cent alcoholic KOH to the extract and allowing the solution to stand in the dark for at least 15 minutes.

The Skelly B phase was obtained by the addition of 40 ml. of water. Following re-extraction of the alcoholic phase twice with 25 ml. portions of Skelly B, the combined Skelly extracts were washed five times with distilled water. The extract was evaporated down, and the final traces of moisture were removed under vacuum. Ten to 15 ml. of Skelly B were added immediately to the dried pigments which now were ready to be chromatographed. The adsorbent employed in the column was a 1:1 mixture of MgO (Micon Brand, no. 2641, Westvaco Corp., Newark, Cal.) with Hyflo Super-Cel (Johns Manville). Following adsorption, the pigments were eluted with a 2 per cent solution of dry acetone in Skelly B. The pigment passing through the column was considered as carotene, and the carotene content of the sample was calculated by standard procedures using pure β -carotene as the reference standard.

The data secured in this experiment are shown in table 5. The striking observation made on these samples was the rancid odor and bleached color in the non-waxed carton containing the trace elements and the total absence of these characteristics when the oxygen was excluded by waxing. When the oxygen was excluded, the greenish color and clean pleasant aroma persisted in all the samples, although with 15 per cent of water the color was slightly olive green.

TABLE 5

Effect of water level, temperature, trace elements, on carotene, color, aroma and pressure of mixed feed with 15 per cent alfalfa stored at 33 to 36° C.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
<i>No trace elements, Fe, Cu, Mn, Co</i>						
No seal	Bleached	None	3.6	39.6	2.7	93.2
Sealed	Green	None	2.3	39.6	13.7	65.4
Sealed	Green	None	5.0	39.6	16.6	58.1
Sealed	Green	None	7.5	39.6	34.4	13.1
Sealed	Green	None	10.0	39.6	40.8	0.0
Sealed	Green	None	12.5	39.6	41.0	0.0
Sealed	Slight olive green	Positive	15.0	39.6	42.6	0.0
<i>Plus trace elements, Fe, Cu, Mn, Co</i>						
No seal	Bleached*	None	4.3	39.6	1.1	97.3
Sealed	Green	None	3.2	39.6	3.8	90.5
Sealed	Green	None	5.0	39.6	6.9	82.5
Sealed	Green	None	7.5	39.6	27.0	31.8
Sealed	Green	None	10.0	39.6	41.8	0.0
Sealed	Green	None	12.5	39.6	46.0	0.0
Sealed	Slight olive green	None	15.0	39.6	46.3	0.0

*Rancid aroma; all other samples had a pleasant aroma.

In the series without the trace elements, the unwaxed material was bleached but possessed a clean non-rancid aroma. In the waxed samples, the greenish color and pleasant aroma persisted in all the samples, although with 15 per cent of water the product was slightly olive green with a slight fermentation aroma.

Involved in the development of the process of carotene preservation in the materials investigated is the question of an economical, practical airtight re-

TABLE 6

Record of preservation of the carotene of dehydrated alfalfa in single thicknesses of Saran tubes (200 gauge). Stored at 33 to 36° C. for 2 months.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
No seal	Green	None	4.2	377	127	67.0
Sealed*	Green	None	7.5	377	368	2.3
Sealed	Green	None	7.5	377	364	3.4
Completely waxed	Green	None	7.5	377	366	2.8
Sealed	Green	None	10.0	377	372	1.3
Sealed	Green	None	10.0	377	346	8.0
Completely waxed	Green	None	10.0	377	372	1.3
Sealed	Olive green	None	12.5	377	363	3.7
Completely waxed	Olive green	None	12.5	377	361	3.9

* Sealed refers to Saran tubes, waxed only at the end tube joint.

ceptacle. Materials such as tin and iron would be available, but prices probably would prohibit their general use. Sheet aluminum has been tried but is liable to have pin holes and was not effective. Fiber cartons allowed air transmission and gave negative results even when lined with asphalt or paraffin paper liners. Waxed cartons were very effective but the process of waxing did not seem practical. Bags made with Kraft paper treated with Melamine resin were not effective. Among the plastics, polyethylene pouches in Kraft paper bags were tried but found ineffective. The oxygen and carbon dioxide transmission rates of cellophane, nylon, parafilm, pliofilm and polyvinyl alcohol were considered too high to warrant a trial.

Saran, a vinyl-vinylidene chloride copolymer manufactured in various thicknesses was investigated. The 200 gauge material was made into tubes about 1 foot long and sealed at the ends and seams with Flexowax. These tubes were filled with dehydrated alfalfa with varying water content (7.5, 10 and 12.5 per cent) and stored at 33 to 36° C. for 2 months. The records with Saran are shown in table 6. The carotene losses were practically zero. While no claim is made that this material is absolutely negative to oxygen and carbon dioxide transmission, yet the rates of transmission must be very low, even where the alfalfa contained 15 per cent of water. Methods of using this material for the production of a bag or a carton suitable for use in the dehydrated alfalfa and dehydrated cereal grass industry are in progress. It also would appear that such an oxygen-impervious bag or receptacle would find large application in the feed and food industry, where exclusion of oxygen from materials surrounded by an inert gas such as carbon dioxide or nitrogen is desirable.

DISCUSSION

The data on the four dehydrated materials show that a definite level of water with exclusion of oxygen can preserve the carotene of these materials. In a previous study (3), the authors demonstrated the effect of moisture upon the rate of respiration in dehydrated materials. A water content of 5 to 7 per cent or lower apparently does not allow a sufficiently rapid rate of oxygen utilization to prevent significant carotene destruction. Dehydrated materials containing 7.5 per cent or more of moisture when sealed showed good carotene preservation; hence, it is concluded that the oxygen tension in such samples is reduced to a low level in a period of a few days. When the water level is above 10 per cent, the partial destruction of chlorophyll generally supervenes. This is especially true when the storage temperature is as high as 33 to 36° C. for 3 months. Shorter periods of storage at such high temperatures may not affect seriously the green color. Since a green color of the product is much prized by the trade, a water level of about 10 per cent under sealed conditions is recommended to achieve a high preservation of the carotene and maintain the green color.

One must expect variation in the behavior of these dehydrated plant materials to the process outlined. Early harvested materials may have a different rate of respiration than those harvested late in the season. A leaf meal would be

expected to behave differently than a meal composed of both leaf and stem. The season's rain fall, the latitude, the type of soil and the method of dehydration all may have their influence on the behavior of these plants under storage. The length of the period between harvesting and storage also may be an important factor. These problems might well be studied.

The results on carotene preservation in a mixed feed under sealed conditions, with or without trace elements, are especially interesting. That the carotene from only 15 per cent of alfalfa in a mixed feed can be preserved when the oxygen is excluded is important information. It is believed that not only does the alfalfa respire and use up the oxygen when there is a proper moisture content, but that other plant materials also will respire and supplement the activity of the alfalfa. However, this point has not been proven definitely, but since the carotene was preserved in a mixed ration containing only 15 per cent alfalfa (principal carotene source), it is logical to conclude that other plant tissues are contributing to the respiration.

The green color was well preserved when the mixed feed was sealed while the unsealed product became distinctly bleached. Further, in the presence of the trace elements, the unsealed material developed a definite rancid odor, a condition that did not develop in the absence of the trace elements under sealed and unsealed conditions. Consequently, it would seem unwise to add these trace elements to a mixed feed that is to be stored for months and where free access to oxygen is allowed. Some other vehicle, probably common salt, should be used for providing additional trace elements when needed by our livestock.

Many trials were made of materials presumed to be airtight. It is imperative that receptacles for carrying out the outlined process for carotene preservation be airtight, that is, made of materials that will retain the carbon dioxide generated within and prevent the transmission of oxygen into the receptacle. Flexowax was effective but probably impractical. There may be other suitable waxes, but this was the only one tried. Among the plastic films, Saran (Dow Chemical Company) possessed a high preservation quality. It has a high tensile strength and should lend itself to the solution of the problem involved in these studies. Other suitable plastic films may be found.

In practice it is correctly assumed that storage of feed materials with a high water content may lead to the growth of molds and even spontaneous combustion. Both conditions are governed by access to oxygen. With a process that excludes oxygen or greatly lowers its tension, common molds cannot grow and combustion cannot start.

SUMMARY

1. The effect of moisture level and temperature on carotene losses in dehydrated alfalfa and cereal grasses was studied under sealed conditions. The moisture levels studied were 2.5 to 15 per cent and the temperatures employed were 22 to 25° C. and 33 to 36° C.

2. In most instances, almost complete carotene preservation resulted with 10 to 15 per cent of water. Preserving both the carotene and the green color

was best accomplished at 7.5 to 10 per cent of water with 10 per cent as the preferred level because of the more optimum carotene preservation with no detrimental color change. At 7.5 per cent of water, the amount of loss was unpredictable and varied from 2.5 to 17 per cent. The losses increased with decreasing water levels below 7.5 per cent and at 2.5 to 5 per cent varied from 5 to 32 per cent.

3. Storage at 22 to 25° C. (room temperature) was more favorable for the preservation of the green color at 10 to 15 per cent of water level than storage at 33 to 36° C. Little difference in color preservation was observed at either temperature with the moisture below 10 per cent. Postitive pressures seldom were observed with 10 per cent moisture or less and storage at 22 to 25° C.

4. Storage under sealed conditions at 33 to 36° C. of a mixed feed containing 15 per cent alfalfa as the main source of carotene resulted in complete carotene retention with 10 per cent of moisture. Below 7.5 per cent the losses were large. The feed became bleached in the unwaxed carton but retained a pleasant aroma. In waxed cartons feed at any moisture level remained green and had pleasant aromas.

5. Where the mixed feed contained the added trace elements Fe, Cu, Mn and Co, the contents of the unwaxed carton were bleached and also possessed a rancid or tallowy odor. Under sealed conditions the green color and fine aroma were retained, and at 10 per cent and above of water, the carotene was preserved completely.

6. Investigation of many materials as barriers to oxygen and carbon dioxide transmission finally led to the use of Saran, a plastic film. It was found effective for the preservation of carotene in dehydrated alfalfa, with a proper water level.

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STUDIES ON RUMINAL GAS FORMATION AND ON CONSUMPTION OF ALFALFA PASTURE BY CATTLE

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This paper is concerned with the effect of diet on ruminal gas formation and with studies on the consumption of legume pasture. It was shown earlier (2) that no more gas was formed on a bloat-provoking diet such as green alfalfa tops than on a non-bloat-provoking diet such as alfalfa hay and grain. This observation led to the conclusion that increased gas production in itself could not explain acute bloat. In the same paper, it was shown that the rate of gas production depended upon the amount of feed consumed. Furthermore, both the present authors (2) and Quin (6) have shown by introducing gas into the rumen that much more can be expelled by belching than ever is produced in the rumen. Consequently, the hypothesis that acute bloat is due to a lack of sufficient coarse roughage in the rumen to induce eructation has been suggested (2). With this hypothesis, the rate of gas production is still an important consideration because belching rarely, if ever, is completely inhibited on diets low in coarse roughage. On the basis of this theory, it has been possible to induce and prevent bloat at will (3). Nevertheless, fatal bloat is not always produced on all succulent fields, and this failure has appeared to be due to a low consumption of alfalfa.

Quin (6) has suggested that bloat depends on both a high sugar content of the legumes at certain times which accelerates gas production, and a high saponin content which results in foaming with a consequent trapping of the gas and which thus prevents eructation. In support of the importance of the first factor, he submits evidence that glucose added to ruminal contents speeds up gas production more than does the addition of starch. The second postulate was based on his observation that ruminal ingesta from animals fed on alfalfa had a greater tendency to foam than ingesta from animals on other feeds, and on the report of Jacobson (4) that alfalfa contains a saponin with strong foam-producing properties. The results reported in the present paper confirm Quin's finding that glucose, under certain conditions, results in a more immediate increase in gas production than does starch, but further work is necessary to establish the view that changes in sugar content play a major role in determining the incidence or severity of bloat. The present authors have stuck a number of bloated cattle and find that foaming is not the cause of many cases of bloat. In one animal near death, the excess gas easily escaped when the animal was stuck with a trocar cannula, and in another severely bloated animal the excess gas was withdrawn by means of a stomach tube without obstruction by foaming. However, foaming may prevent eructation under certain conditions.

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EXPERIMENTAL

Dairy cows, in most instances lactating Jerseys, were used in the present studies. The method used for determining the rate of gas production has been described (2) as also has the method for determining food consumption on pasture (1). The rumen was tapped for gas production by means of a trocar cannula intended for bleeding horses.

Ruminal Gas Production Studies

Comparison of gas production on green alfalfa tops and on green Sudan grass. Although the rate of gas production on non-bloat-provoking diets such as alfalfa hay and grain has been compared to that on green alfalfa tops,

TABLE 1
*Comparison of ruminal gas formation following feeding of Sudan and alfalfa tops.
The cows were fed ad libitum throughout the 4-hour experimental period*

Cow no.	Pounds of Sudan or alfalfa tops consumed ^a	Cubic feet of gas formed:		
		Half-hour before feeding	Half-hour after feeding	First 4 hours after feeding
		Sudan tops		
760	69.7	0.08	0.28	4.02
760	50.4	0.26	0.47	5.43
757	7.5	0.32	0.47	2.77
760	63.0	0.34	0.59	6.70
Av.	47.6	0.25	0.45	4.73
		Alfalfa tops		
760	26.9	0.06	0.48	4.17
757	2.4	0.34	0.28	3.29
760	19.7	0.30	0.78	6.82
832	12.9	0.34	0.43	3.00
Av.	15.5	0.26	0.49	4.32

^a In addition to the tops, cow 760 received 4 pounds of a concentrate mix the night before the trial. Cows 757 and 832 received 4 pounds of concentrates the night before and the morning of the trial.

grasses have not been compared with legumes. Because bloat rarely occurs on grasses, this comparison seemed desirable.

The alfalfa and Sudan tops were cut and fed in the barn. The cows were pastured on the field from which the tops were to be taken for two days preceding the trial. No hay was fed the night previous or on the morning of the trial, but the regular concentrate allowance was given (4 to 8 lb. per day, depending on milk production). Gas production was determined over a 30-minute control period before feeding the alfalfa or Sudan. The object of the experiment was to determine the amount of gas formed on the two feeds when cows were given free access to the feed over a 4-hour experimental period. The results are shown in table 1. The gas formation during the first 4 hours after the beginning of feeding was approximately the same for the two feeds, but the average consumption of Sudan was three times that of alfalfa. Conse-

quently, it appears that the rate of gas production would be greater with alfalfa than with Sudan if equal quantities were fed.

Cow 757 ate very little during the gas determination period, as she was distressed by the presence of the cannula. Cow 760, on the other hand, evidenced no discomfort upon insertion of the trocar cannula; she continued to eat and ruminate in a normal manner. These individual differences are mentioned to point out that the use of the trocar cannula in gas production studies necessitates some discrimination in the selection of suitable experimental subjects.

It may be noted that there is an increase in gas production during the first 30 minutes after feeding (table 1). The promptness of acceleration of ruminal gas formation following ingestion of feed is an interesting phenomenon. On a given feed, there are some discrepancies between the amount consumed and the volume of gas formed which are difficult to explain. The amount of feed consumed was not measured on the 2 days preceding the test, and it may be that variations in the volume of ingesta present in the rumen at the beginning of the trial may provide an explanation.

TABLE 2

Comparative effects of glucose and starch on ruminal gas formation in cows given free access to green alfalfa tops for 4 hours preceding the experimental period. Two kg. of starch or glucose in 6 liters of H₂O were administered through a cannula directly into the rumen

Cow no.	Drench	Cubic feet of gas formed:		
		Hour before drench	Hour after drench	Second hour after drench
760	glucose	1.50	1.56	1.69
832	glucose	0.73	0.85	0.76
	Av.	1.12	1.21	1.23
760	starch	1.14	1.14	1.53
760	starch	1.45	1.39	1.34
	Av.	1.30	1.27	1.45

Comparative effects of glucose and starch on gas formation. In the light of Quin's hypothesis and data cited above, it seemed desirable to obtain more information on the effects of glucose and starch on gas formation. The tests were run under two conditions: in the first, the cows were given free access to green alfalfa tops fed in the barn for 4 hours preceding the test period; in the second, the cows were fed 9 lb. of alfalfa hay and 6 lb. of rolled barley 20 hours before the experimental period. Gas production was determined for 1 hour before the experimental period in the first experiment and for 30 minutes in the second. To introduce the test substance, glucose or starch, a rubber tube with a funnel attached to one end was connected to the side arm of the cannula. During the introduction of the test substance, the tube leading from the cannula to the gas meter was clamped off. The solution of starch or glucose was poured into a funnel elevated 3 or 4 feet above the level of the entrance of the cannula into the rumen, the fluid flowing into the rumen by gravity. Five to ten minutes were needed in introducing the solution.

In table 2 is shown the effect of administering starch or glucose to cows fed alfalfa tops for 4 hours preceding the test period. No significant change in the rate of gas formation resulted with either glucose or starch.

The results obtained when the cows were fed 20 hours before the experimental period are given in table 3. The amount of glucose or starch administered was reduced from 2 kg., as in the previous experiment, to 1 kg., because the higher dose of glucose on a partially empty rumen had an adverse effect on the cow, resulting in diarrhea and loss of appetite. Under this regime, 1 kg. of glucose in 3 liters of water increased gas production regularly within the first half hour after its introduction. The response to an equal amount of

TABLE 3

Comparative effects of glucose and starch on ruminal gas formation in cows fed 20 hours before the experimental period. One kg. of starch or glucose in 3 l. of H_2O was administered through a cannula directly into the rumen

Date of trial	Cubic feet of ruminal gas formed:					
	Hour before drench ^a	1st hour after drench	2nd hour after drench	3rd hour after drench	4th hour after drench	Total after drench
1 kg. glucose administered						
Mar. 11	0.33	0.67	0.68	0.51	0.53	2.39
Mar. 23	0.31	1.11	1.11	0.78	0.76	3.77
Mar. 30	0.42	1.09	0.85	0.85	0.52	3.31
Apr. 13	0.44	1.00	0.96	0.83	0.45	3.24
Av.	0.38	0.97	0.90	0.74	0.57	3.18
1 kg. starch administered						
Mar. 18	0.56	0.80	0.94	0.84	0.56	3.13
Mar. 25	0.48	0.45	0.62	0.67	0.56	2.29
Apr. 6	0.48	0.43	0.76	0.98	0.81	2.98
Av.	0.51	0.56	0.77	0.83	0.64	2.80

^a Gas was determined for only 0.5 hr. before drenching. The figure obtained was multiplied by 2 to facilitate comparison of gas production before and after drenching.

starch was not marked until the second hour after drenching, but during the third and fourth hours more gas was liberated than with glucose. The tests on starch and glucose were run for 30 minutes longer than is shown in table 3. During this last half hour, there was an average production of 0.22 cubic feet of gas with glucose and 0.32 cubic feet with starch. Thus the effect of starch is more prolonged and the total gas produced is apparently the same as with glucose.

Studies on Consumption of Alfalfa Pasture

For these studies, the cows were weighed in and out of pasture and during the intervening period all excreta were collected and weighed. The pasturing period extended from 8 a.m. to 2:30 p.m. The studies were made between June 14 and October 10. Insensible losses were determined on 2 days and amounted to approximately 3 lb. per hour, but the insensible losses were not taken into account in calculating feed consumption. On a few occasions, the

insensible losses exceeded feed consumption, thus explaining the apparent negative consumption values shown in figures 1 and 2.

One of the objectives of the experiment was to determine if palatability varied in different fields. Further, it was desired to ascertain the influence of maturity on palatability. Decisive answers were not obtained to either question for reasons which will be explained. The results of the study are summarized in figures 1 and 2.

Figure 1 gives the feed consumption on two different fields, 1-C South and Dairy Field 4. Two lactating cows were used in this part of the study; cow

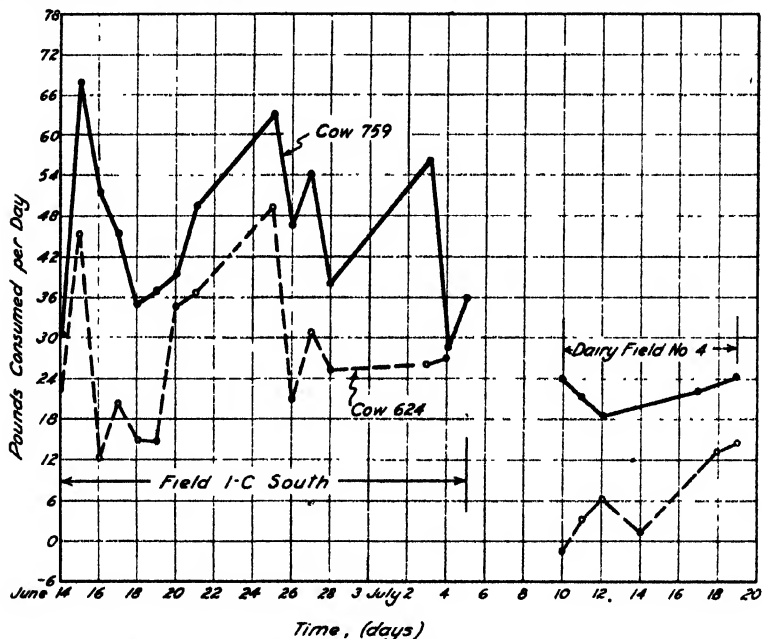


FIG. 1. Consumption of alfalfa on fields 1-C South and Dairy Field 4. The cows, 759 and 624, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

624 received 5 lb. of a concentrate mix at 3 a.m. and 3 p.m. and cow 759 received 7 lb. night and morning. Cow 759 had been shown to be susceptible to bloat in previous studies, whereas cow 624 never had bloated severely, even under conditions in which the majority of the herd had bloated. No hay was fed. The cows were pastured intermittently on 1-C South from June 14 to July 5. There were 12 acres in this field, and thus the amount consumed by the 2 cows had no appreciable influence on the amount of feed available during this period. Although the stand of alfalfa on 1-C South appeared to be fairly clean on cursory examination, there were some weeds and annual grasses on the irrigation checks. When the cows were first put on the field, the alfalfa was about 1 foot tall and very succulent. Furthermore, the alfalfa was relatively

unpalatable and during the first week the cows ate approximately as much weeds and grasses as alfalfa. One cow did not bloat on this field, and the other, cow 759, bloated slightly on 3 different days. We attribute this relative lack of bloat to the consumption of sufficient weeds and grasses to induce belching. In support of this view is the fact that both cows ruminated more than one would expect when pasturing on succulent alfalfa without access to hay. Cows ruminate very little on fields causing severe bloat. Experience in the next field, Dairy Field 4, with the same cows adds weight to this interpretation. This field had been pastured earlier in the season and was devoid of contaminating

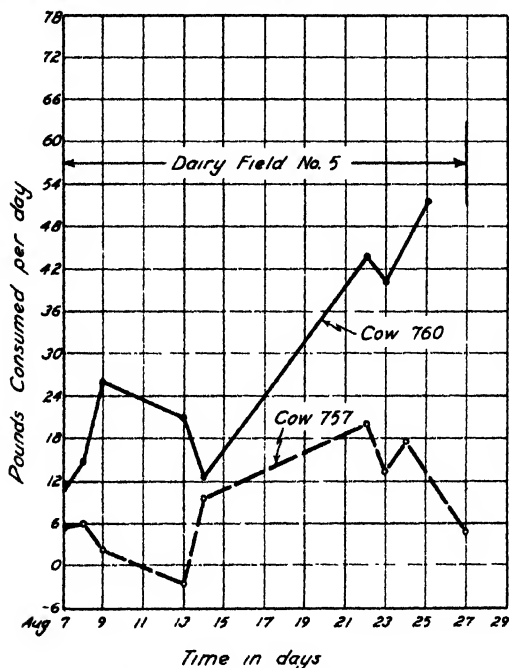


FIG. 2. Consumption of alfalfa on Dairy Field 5. The cows, 760 and 757, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

weeds and grasses. Cow 624 bloated on 4 of 6 pasturing days on this field, and cow 759 bloated every day—on two occasions the bloat was sufficiently severe to require treatment with turpentine. "Severe bloat" refers to a condition in which there is marked distress, frequent urination and defecation and a ruminal pressure of 45 to 70 mm. Hg (1).

Palatability of the alfalfa on the two fields was considered. Field 1-C South was pastured over a 3-week period during which the alfalfa became progressively more mature. It was in the late bud stage at the termination of the trial. This field was irrigated on June 11. The fact that the cows ate a considerable proportion of weeds, particularly during the first week, presents

some difficulties in interpretation. However, the cows were under constant observation during the pasturing period by the attendants collecting the excreta, and there is little doubt but that the cows ate a greater amount of alfalfa as it became more mature. Furthermore, there is little doubt that the alfalfa in field 1-C South was more palatable than in Dairy Field 4. The results are difficult to evaluate, however, for two reasons: on field 1-C South, the exact consumption of alfalfa is unknown because the cows ate grasses and weeds in addition to alfalfa; secondly, the consumption of alfalfa on Dairy Field 4 was depressed as the result of bloat. In other words, when the cows bloated, they stopped eating.

The results on Dairy Field 5 with cows 760 and 757 are shown in figure 2. Here the evidence seems a little more clear-cut that the alfalfa becomes more palatable as it matures. On August 13 and 14, cow 760 bloated, on the latter date sufficiently severely to require treatment with turpentine. This explains her relatively low consumption on these days. By August 22nd, the alfalfa was in the early bloom stage and was sufficiently coarse to induce frequent rumination. No adequate explanation is available for the relatively low and sporadic feed consumption of cow 757.

DISCUSSION

The results on ruminal gas formation following feeding of green Sudan and alfalfa tops indicate that one might expect a greater amount of gas formed from alfalfa if equal amounts of the two feeds were given. With *ad libitum* feeding, the total gas formed from the two feeds, however, was approximately the same because of the greater consumption of Sudan. Increased gas production on legumes does not in itself provide an adequate explanation of bloat; cows will bloat on amounts of alfalfa comparable to those consumed in these experiments but bloat did not occur when normal animals were given an amount of Sudan producing an equivalent volume of gas. Previous studies (2) have shown that dry legume hay results in as much gas production as green alfalfa. These data give further confirmation, therefore, that it is the inability of animals to eructate the gas on legumes which makes alfalfa and clover dangerous from a standpoint of bloat. Nevertheless, the rapid gas formation on legumes undoubtedly is a contributing factor in bloat.

Quin (5) has compared the rate of gas formation on alfalfa and grass hay. He reports a rapid production of gas on alfalfa hay, a result in accord with our studies (2). On the contrary, he found no gas formed over a 90-minute period in two of three trials with sheep on a basal diet of grass hay. In the light of the data reported herein on a green grass (Sudan), this result needs further confirmation.

When cows were fed 20 hours before the experimental period, glucose caused an earlier increase in gas formation than did starch, but the total gas produced from the two substances over a period of 4.5 hours was about the same. Quin reported that when starch, in the form of maize, was given to sheep maintained on a basal diet of green alfalfa, there was no gas formed over a 90-minute

period. When cows were given a full feed of alfalfa during a 4-hour interval preceding the test period, no difference in gas formation between glucose and starch was observed. Conceivably the sugar content of alfalfa could be a contributing factor in bloat as postulated by Quin, but further studies are necessary to establish the point.

The present studies on palatability of legumes at different stages of maturity were not conclusive but indicated that alfalfa increases in palatability as it matures. Two main difficulties in these studies were encountered: first, cows ate weeds and grasses along with the alfalfa when the fields were contaminated; second, cows on pure alfalfa stands bloated and this in turn depressed feed consumption and made it impossible to obtain a true estimate of palatability. Therefore, it appears that a more desirable procedure would be to cut the alfalfa tops and feed them in the barn. In this way, the weeds and grasses could be avoided. Further, it would appear necessary to supplement the diet with sufficient Sudan grass hay or with green Sudan to obviate bloat.

SUMMARY AND CONCLUSIONS

In an average of four trials, 4.7 cubic feet of gas were produced when cows consumed 47.6 lb. of green Sudan tops fed *ad libitum* over a 4-hour period as compared to 4.3 cubic feet when cows consumed an average of 15.5 lb. of green alfalfa tops over a similar period.

The amount of gas formed following drenching with glucose or starch was determined both by feeding cows 20 hours before the experimental period or feeding them with alfalfa tops *ad libitum* 4 hours preceding drenching. In the former instance, glucose caused a more prompt increase in gas formation, whereas the effect of starch was more prolonged. When cows were fed immediately preceding drenching, on the other hand, no difference between glucose and starch as regards gas formation was discernible.

Studies on the consumption of alfalfa pasture indicate that alfalfa becomes more palatable as it matures up to the early bloom stage, but the results were inconclusive.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAI- ARETIC ACID IN DAIRY PRODUCTS. II. ITS ANTIOXYGENIC PROPERTIES IN UNSWEETENED FROZEN CREAM¹

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The use of antioxidants in retarding the development of oxidized flavor during the storage of frozen cream has been studied by several investigators (2, 3, 4, 5, 6). The work reported herein consists of a study in which nordihydroguaiaretic acid (NDGA) was used to retard the development of oxidized flavor during the storage of unsweetened frozen cream containing 40 per cent milk fat.²

EXPERIMENTAL PROCEDURE

Two grades of cream, one of high and the other of low quality, were used in this study. Standard plate count (1), acidity and pH were the criteria upon which quality was based (table 1).

TABLE 1

The standard plate count, titratable acidity and pH of the raw and pasteurized cream

	<i>High quality</i>		<i>Low quality</i>	
	(a)	(b)	(c)	(d)
<i>Raw cream</i>				
Standard plate count	340,000	32,000	345,000,000	6,000,000
Titratable acidity, as % lactic acid	0.120	0.140	0.155	0.125
pH (25° C.)	6.74	6.63	6.50	6.66
<i>Pasteurized cream</i>				
	<i>150° F.</i>	<i>170° F.</i>	<i>150° F.</i>	<i>170° F.</i>
Standard plate count	900	55	2,000	2,860
Titratable acidity, as % lactic acid	0.125	0.135	0.145	0.140
pH (25° C.)	6.62	6.60	6.50	6.39

The different batches of cream were standardized to contain 40 per cent milk fat. NDGA was added after pasteurization as a 10 per cent solution in glycerol or as a 5 per cent water suspension. The concentrations of NDGA were computed on the basis of the fat content of the cream. When used, copper was added at a concentration of 0.5 p.p.m. in the form of a 0.5 per cent aqueous solution of copper sulfate.

The cream was pasteurized in well-tinned equipment, cooled to 40–45° F., sealed in tinned cans holding 300 ml. and stored at –12 to –20° F. For monthly flavor criticisms, the frozen cream was thawed by holding it 24 hours at 40° F.

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¹ The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science, 1947.

² The legality of adding antioxidant material to dairy products would need to be established before its use could be recommended. The authors' interest in the product studied was mainly scientific, although the practicable possibilities of a study of this nature always must be recognized.

TABLE 2
The antioxidant effect of NDGA added to unsweetened cream stored at sub-zero temperatures

Treatment	Flavor criticisms									
	1 mo.		3 mo.		5 mo.		7 mo.		9 mo.	
	(1) ^a	(2) ^b	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
<i>Pasteurized at 150° F. for 30 min.</i>										
Series A. Low quality cream. No copper added.										
Control							1 ^d	1	1	2
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series B. Low quality cream. 0.5 p.p.m. copper added.										
Control			4	4			5 ^c	5 ^c	5 ^c	5 ^c
Control + 0.00125% NDGA			1	1			2	2	4 ^c	4 ^c
Control + 0.005% NDGA			1	1			2	2	4 ^c	4 ^c
Series C. High quality cream. No copper added.										
Control										
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series D. High quality cream. 0.5 p.p.m. copper added.										
Control			3	4			5	5	5	5 ^c
Control + 0.00125% NDGA			±	1			2	2	2	2
Control + 0.005% NDGA			±	1			1	1		
<i>Pasteurized at 170° F. for 15 min.</i>										
Series E. Low quality cream. No copper added.										
Control			±	1					±	1
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series F. Low quality cream. 0.5 p.p.m. copper added.										
Control			±	±					±	±
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series G. High quality cream. No copper added.										
Control										
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series H. High quality cream. 0.5 p.p.m. copper added.										
Control										
Control + 0.00125% NDGA										
Control + 0.005% NDGA										
Series I. High quality cream. 0.5 p.p.m. copper added.										
Control										
Control + 0.00125% NDGA										
Control + 0.005% NDGA										

^a Flavor judged immediately after taking cream out of storage and thawing it.

^b Flavor judged after thawing.

^c No oxidized flavor present.

^d The numbers 1 to 5 indicate increasing levels of oxidized flavor defect.

It then was held at 40° F. for 1 week after which it was judged again for flavor. The judging panel was composed of three or more persons.

RESULTS

The data in table 2 are typical of results obtained with cream which was placed in storage during the months of August and September. There were no significant differences in the antioxygenic effectiveness of the NDGA when added in glycerol solution and when added in a water suspension. Therefore, the data presented in table 2 includes only results from cream treated with NDGA in glycerol solution.

The effect of concentration of NDGA. A concentration of 0.005 per cent NDGA was more effective than one of 0.00125 per cent. This is demonstrated in the results obtained with the high quality cream which contained added copper and which was pasteurized at 150° F. for 30 minutes (table 2, series D). Oxidized flavor had developed at the end of 3 months storage in the control sample. While this off-flavor had developed at the end of 5 months in the cream treated with both 0.00125 per cent and 0.005 per cent NDGA, the intensity of the off-flavor at the end of 7 months was less in the cream containing 0.005 per cent than in the cream containing 0.00125 per cent NDGA.

The effect of pasteurization temperature. Development of oxidized flavor was retarded by pasteurizing the cream at 170° F. for 15 minutes. Oxidized flavor had developed at the end of 7 months storage in the control sample of series A which was pasteurized at 150° F. for 30 minutes, while it did not develop during storage at sub-zero temperatures in the similar cream pasteurized at 170° F. for 15 minutes. Oxidized flavor was present at the end of 1 month in the control sample of series B which was pasteurized at 150° F. for 30 minutes, whereas it did not develop in the similar cream pasteurized at 170° F. for 15 minutes.

There was no oxidized flavor development during storage for 11 months at sub-zero temperatures in the high quality cream which contained no added copper (table 1, series C and G). The off-flavor was present at 3 months in the control sample of series D which was pasteurized at 150° F. for 30 minutes but was not detected until the end of 5 months in the similar cream pasteurized at 170° F. for 15 minutes. While the keeping quality of the cream pasteurized at 170° F. for 15 minutes was superior to that pasteurized at 150° F. for 30 minutes, it had a cooked flavor which persisted throughout the storage period. The keeping quality of the cream which was pasteurized at 150° F. for 30 minutes and which contained NDGA but no added copper was comparable to that of cream pasteurized at 170° F. for 15 minutes.

The effect of quality of the cream. The cream which was pasteurized at 150° F. for 30 minutes developed the oxidized flavor in the control sample containing added copper (series B) at the end of 1 month, but the off-flavor was not detected in the similar high quality cream until the end of 3 months (series D).

There was no oxidized flavor development in any of the low quality cream pasteurized at 170° F. for 15 minutes during storage for 11 months at sub-zero

temperatures. However, the off-flavor was present at the end of 5 months in the control sample of the similar high quality cream containing added copper (series H).

The effect of quality as indicated in this study was variable. The high quality cream which was pasteurized at 150° F. for 30 minutes had a better keeping quality than the similar low quality cream with respect to the oxidized flavor development. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.

The effect of holding the thawed cream at 40° F. for 1 week. Oxidized flavor developed frequently in the control samples which were held at 40° F. for 1 week, although they did not have the off-flavor when they were taken out of storage. This relationship was illustrated in the control sample of series D pasteurized at 150° F. for 30 minutes which had been stored for 1 month at sub-zero temperatures and did not have the oxidized flavor when first removed from the low temperature storage, but developed it after the sample had been held at 40° F. for 1 week. The same observation was made after 5 months in the control samples of series E and after 7 months in the control samples of series H, both of which were pasteurized at 170° F. for 15 minutes.

After storage for 1 week at 40° F., the oxidized flavor usually increased in intensity in the control samples which had that off-flavor when they were first taken out of storage. This is evident in the cream pasteurized at 150° F. for 30 minutes in the control samples of series A after 9 and 11 months, in series B after 1, 3 and 5 months and in series D after 5 months. This trend also was evident after 7, 9 and 11 months in the control samples of cream in series H which had been pasteurized at 170° F. for 15 minutes. However, the intensity of the oxidized flavor did not increase during storage at 40° F. in the samples which contained NDGA.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.005 per cent nordihydroguaiaretic acid were found to retard the development of oxidized flavor in unsweetened frozen cream during storage for 11 months.

2. In the absence of added copper, the keeping quality of the cream which contained nordihydroguaiaretic acid and was pasteurized at 150° F. for 30 minutes was comparable to that pasteurized at 170° F. for 15 minutes but to which the antioxidant had not been added.

3. In this study, the high quality cream pasteurized at 150° F. for 30 minutes had a better keeping quality than the low quality cream similarly pasteurized. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.

4. During storage for 1 week at 40° F., an oxidized flavor developed frequently in the control samples, although these samples did not have the off-flavor when they were taken out of storage at sub-zero temperatures. This did not occur in the cream which contained nordihydroguaiaretic acid.

5. During storage for 1 week at 40° F., the intensity of the oxidized flavor usually increased in the control samples which had the off-flavor when they were

first taken out of storage at sub-zero temperatures. This did not occur in the oxidized samples which contained nordihydroguaiaretic acid.

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THE RELATION BETWEEN THE MONTH OF CALVING AND YEARLY BUTTERFAT PRODUCTION¹

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Approximately 71 per cent of the dairy cows of Oregon are located west of the Cascade Mountains; and about two-thirds of these, or 46 per cent of the total, are found on farms in the ten Willamette Valley counties, a region with an average temperature of 52° F. and a monthly mean range from a high of 65° F. in July to 38° F. in December. The average rainfall of about 42 inches comes mostly during the winter months. Since the climate under these conditions is mild and does not show large seasonal variations in comparison with some other parts of the United States, it became of interest to study the effect of the month of calving on butterfat production.

Differences in yearly milk production between cows freshening in the different months of the year, in various parts of the United States, have been found to exist (1, 2, 5, 7, 8). The season of the year in which the cow freshens also was reported to exert an effect on her butterfat production (1, 4, 8). In Connecticut, Frick *et al.* (2) found that the differences in milk production of cows calving in the different months of the year were highly significant statistically.

PROCEDURE

Data for the present study were obtained from the record books of the dairy herd owned by Oregon State College and from official test records of cows tested in Oregon covering the years 1910 through 1946. Only first-calf, 2-year old records were used. The official records of butterfat production were tabulated separately for cows milked twice a day during 305-day and 365-day lactations. Production of cows milked three times daily, part or all of the milking period, was reduced to a 2-times a day milking basis by using the factor 0.0655 of 1 per cent for each day the cow was milked 3 times. The distribution of the 2690 records between breeds was 1881 Jerseys, 358 Guernseys, 301 Holstein-Friesians and 150 Ayrshires. The number of records available from the College herd was 359, while 2331 were from private herds.

An analysis of variance (6) was applied to the data to find out the significance of the difference in butterfat production of cows calving each month of the year.

RESULTS AND DISCUSSION

Information on the butterfat records of first calf heifers used in the study is given in table 1.

Table 2 gives a summary of the results of the statistical analysis of the data. The variations in butterfat production among cows freshening within each month

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TABLE 1
Average yearly butterfat production of five groups of first calf heifers

Month of fresh- ening	Group 1 ^a		Group 2		Group 3		Group 4		Group 5	
	No. of heif- ers	Av. prod.	No. of heif- ers	Av. prod.	No. of heif- ers	Av. prod.	No. of heif- ers	Av. prod.	No. of heif- ers	Av. prod.
		(lb.)		(lb.)		(lb.)		(lb.)		(lb.)
January	80	455	34	372	66	488	19	483	42	320
February	78	464	38	390	68	489	23	479	28	295
March	91	469	32	375	64	474	20	477	24	330
April	102	421	26	379	76	473	11	467	25	304
May	72	425	27	380	81	464	13	479	27	372
June	53	438	18	359	62	465	10	458	19	326
July	48	434	26	338	47	458	18	471	22	326
August	69	459	19	384	78	482	23	467	33	322
September	104	446	19	397	105	466	35	472	49	312
October	70	444	25	398	84	450	24	470	29	341
November	64	461	25	389	69	484	9	459	31	329
December	78	445	29	387	72	498	27	474	30	305
Total & mean	909	447	318	379	872	474	232	473	359	323

^a Group 1. Jersey, 305 day, Register of Merit

Group 2. Guernsey, Holstein, Ayrshire, 305 day, Advanced Registry

Group 3. Jersey, 365 day, Register of Merit

Group 4. Guernsey and Holstein, 365 day, Advanced Registry

Group 5. Ayrshire, Guernsey, Holstein and Jersey herd test (college)

were large, and a definite trend was not followed when the monthly averages were studied, but rather an up-and-down line. Jersey cows milked for 305 days were

TABLE 2
Analysis of variance of the butterfat records used in the study

Group	Source of vari- ation	Degrees of freedom	Sum of squares	Variance	Variance ratio	Significance level	
						5%	1%
1	Month	11	209,932.14	19,084.74	2.25	1.80	2.26
Jersey	Error	897	7,594,314.99	8,466.35			
R. of M.	Total	908	7,804,247.13				
305 days							
2	Month	11	77,933.46	7,084.86	1.45	1.82	2.31
Guernsey	Error	306	1,496,925.13	4,891.91			
Holstein	Total	317	1,574,858.59				
Ayrshire							
A.R. 305 days							
3	Month	11	160,275.05	14,570.46	1.02	1.80	2.26
Jersey	Error	860	12,275,470.21	14,273.80			
R. of M.	Total	871	12,435,745.26				
365 days							
4	Month	11	8,700.71	790.97	0.09	1.83	2.34
Guernsey	Error	220	2,009,556.38	9,134.35			
Holstein	Total	231	2,018,257.09				
A.R. 365 days							
5	Month	11	124,238.84	11,294.44	1.71	1.81	2.29
All breeds	Error	347	2,297,328.15	6,620.54			
College	Total	358	2,421,566.99				
Herd Test							

the only group that showed significance at the 5 per cent level, although not significant at the 1 per cent level. Since the other four groups showed insignificant differences between the production of cows freshening in different months of the year, the significance of the first group is of doubtful value.

SUMMARY

The butterfat records of 2690 first-calf heifers in herds located in western Oregon, a region with rather uniformly mild temperature, were studied to determine the effect of the month of calving on yearly butterfat production.

It seems that under western Oregon conditions the season of the year in which a cow freshens has no appreciable effect on her yearly butterfat production.

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SOME FACTORS INFLUENCING THE MALE HORMONE CONTENT OF COW MANURE¹

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Riley and Hammond (8) discovered that the feeding of dried cow manure to day-old chicks caused marked stimulation of the comb growth. Evidence was presented indicating that the factor present was an androgenic rather than a gonadotrophic substance. They reported that "feces from bulls were entirely without effect, whereas feces from pregnant cows, as well as from unbred heifers, had marked androgenic effects."

Turner (10, 11, 12) confirmed the report of the presence of orally-active androgens in the feces of lactating cows when dried at 45° C. The androgen content of the manure of other ruminants, including goats and sheep of both sexes and conditions, was either low or absent. The feces of dairy bulls showed indications of only small androgen excretion by that route.

Gassner and Longwell (1, 4) reported that the concentration of androgens in feces reached a peak during the last week of pregnancy and then dropped sharply to zero at calving. Steer and bull manures were relatively inactive biologically.

The present studies were initiated to throw further light upon the functional relationship between the male hormone eliminated in the feces of dairy cows of the several breeds and reproduction and lactation. Further, in connection with studies concerned with the characterization of the androgens excreted and with methods for their extraction, it was considered helpful to know when the greatest concentration of hormone might be expected.

EXPERIMENTAL PROCEDURE

The fresh manure was collected from individual cows of the Guernsey, Holstein and Jersey breeds in the University of Missouri dairy herd. Complete samples were not collected, rather the feces dropped during the milking period in the morning or afternoon were combined until a sufficient quality was collected for an assay. This usually required 2 to 3 days. Cows in various stages of lactation and pregnancy were included. When a series of samples from the same cow was collected, at least a month intervened between samples. Each collection of fresh manure was placed quickly in a Freas electric drying oven maintained at a temperature of 45° C. Samples were stirred daily. At least 48 hours were required to dry the collection. The dried manure was placed in a large lard can and, when collection was complete, the entire sample was ground in a small hammer mill and thoroughly mixed before assay.

The androgen content of each sample was assayed biologically by the method

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previously described (12). Groups of about 20 sexed White Plymouth Rock chicks were used throughout and the dried cow manure was fed uniformly at the 10 per cent level by substituting the dried manure for an equal weight of alfalfa meal in the basal chick starter ration. These assays were conducted monthly throughout the year.

Each month a group of control chicks and a group fed methyl testosterone at the rate of 20 mg./kg. of feed were included to determine the possible seasonal variation in the responsiveness of the chick comb to androgen. Since no seasonal trend was observed in the average comb weight of either sex in the control group or those fed methyl testosterone, it was decided that no correction factor for season was required (13).

As a measure of the presence of biologically active androgens in the samples, the average comb weight per 100 g. body weight of each sex was determined. The average comb weights per 100 g. body weight for the two sexes were added together and divided by two. This value should represent the average comb weight per 100 g. body weight of a population of chicks containing equal numbers of the two sexes.

As a measure of the biological activity of the male hormone present in the various samples of dried cow manure, comparison may be made with the average comb weight of groups of control chicks of the same age and with groups of chicks fed 10 and 20 mg. of methyl testosterone per kilogram of starter feed. Since the biologically active androgenic hormones present in cow manure are not known, it seems preferable to indicate differences in the various samples in terms of average comb weight rather than in terms of any single androgen. For a comparison of the oral effectiveness of several androgens in fowls the reader is referred to a paper by the writer (14). The average comb weight of all the chicks fed samples of dried cow manure assayed in this study is presented for comparison (table 1).

RESULTS

Effect of pregnancy upon androgen excretion. In the dairy cows available for our study, no clear-cut separation of pregnancy and lactation could be made, since the heifers pregnant for the first time were not stabled. The group of cows included in table 2 were all lactating cows, but they were classified on the basis of the month of pregnancy. Since lactating cows are not bred until 90 days or more after parturition, the group of cows whose manure was assayed during the first month of pregnancy included cows lactating an average of 124 days. By the eighth month of pregnancy, most of the cows had been dried up; only two of eight cows still were lactating slightly. The cows in the ninth month of pregnancy all were dry.

It will be seen that, aside from the second month, the assay results did not vary greatly from month to month until the eighth and ninth months of pregnancy. Apparently there is a tendency for the male hormone excretion rate to increase at the approach of parturition. This rise occurred at a time when the cows were either dry or almost dry.

TABLE 1
Comparison of comb weight of chicks fed control feed, methyl testosterone and cow manure

Treatment	Male chicks					Female chicks					Male + female <div>2</div>	Ratio of control group
	No. of chicks	Av. body weight	Av. testes weight	Av. comb weight	Comb weight <div>100 g.</div> body wt.	No. of chicks	Av. body weight	Av. ovary weight	Av. comb weight	Comb weight <div>100 g.</div> body wt.		
Control	185	(g.) 232.4	(mg.) 48.88	(mg.) 102.69	(mg.) 42.37	199	(g.) 224.8	(mg.) 48.61	(mg.) 51.17	(mg.) 22.76	(mg.) 32.59	1:
10 mg. MTv./kg.	52	238.4	42.86	240.80	96.94	46	236.3	45.45	246.20	104.18	100.56	3.09
20 mg. MTv./kg.	157	239.2	43.61	360.50	150.74	158	236.2	49.78	252.67	111.70	131.22	4.03
10% cow manure	872	251.8	31.47	202.25	80.31	855	236.1	38.10	137.66	58.31	69.31	2.13

* MT = Methyl Testosterone

TABLE 2
Effect of the stage of pregnancy upon the androgen excretion of dairy cattle (assay with white rock chicks—28 days of age)

Month of pregnancy	Lactation days, av.	No. of cows (breed) ^a	Male Chicks				Female Chicks				Male + female $\frac{9}{2}$		
			No. of chicks	Av. body weight (g.)	Av. testes weight (mg.)	Av. comb weight (mg.)	Comb weight 100 g. body wt.	No. of chicks	Av. body weight (g.)	Av. ovary weight (mg.)		Av. comb weight (mg.)	Comb weight 100 g. body weight
1	124	3(2H, 1J)	32	235.1	28.85	147.24	62.63	43	232.6	33.74	106.19	45.65	54.14
2	169	7(5H, 1G, 1J)	64	248.9	29.93	233.48	93.80	67	236.3	38.87	195.93	82.92	88.36
3	180	6(5H, 1J)	69	243.4	32.95	175.53	72.12	48	224.2	51.14	137.18	61.19	66.66
4	216	4(4H)	34	257.4	37.72	153.73	59.72	46	243.9	45.25	111.33	45.65	52.69
5	284	5(2H, 3G)	56	266.1	40.10	161.61	60.73	47	242.2	46.67	102.10	42.16	51.44
6	286	5(2H, 3J)	49	260.5	33.30	210.05	80.63	51	236.6	66.09	124.19	52.49	66.56
7	294	3(2H, 1J)	29	248.0	28.47	148.70	59.96	29	243.6	39.90	142.10	58.33	59.15
8	Almost dry	8(5H, 1G, 2J)	73	234.1	28.45	281.66	120.32	83	224.2	40.41	190.05	84.78	102.55
9	Dry	7(4H, 3J)	73	272.4	32.12	256.81	94.27	69	252.2	37.88	169.31	67.13	80.70
Non-pregnant, dry cows			33	252.6	28.15	205.81	81.48	43	253.3	38.93	168.53	66.53	74.01
Breed													
Holstein	22		226	249.0	32.04	164.63	66.12	219	235.4	43.52	126.40	53.70	59.91
Guernsey	4		49	267.6	37.68	202.67	75.74	38	250.5	45.61	123.66	49.36	62.55
Jersey	7		58	249.4	27.03	233.96	93.81	74	233.6	54.36	165.85	70.99	82.40

^a H = Holstein, G = Guernsey and J = Jersey.

Whether the apparent high level of androgen excretion during the second month is significant is not clear. One of the Holstein cows appeared to excrete an unduly large amount of androgens at this time in comparison with her other assays. Furthermore, since the tabulation according to the stage of lactation shows no similar increase, the writer prefers to believe that this does not represent a general increased level of androgen excretion.

In order to interpret the fluctuation in the androgen excretion rate from month to month during pregnancy due to possible breed variation, the data were classified on the basis of the breed for the first 7 months of pregnancy. The eighth and ninth months were excluded due to possible effect of the preparturient rise in the androgens. This tabulation indicates little difference in the excretion of androgens by Holstein and Guernsey cows, but the Jersey cows appear to excrete greater quantities of androgens under similar conditions.

A small group of non-pregnant dry cows also is included. The relatively higher androgen excretion rate by this group, as compared to the pregnant group, is believed to be due to the presence of a predominant number of Jersey heifers. It would appear that neither pregnancy nor lactation is necessary for the excretion of relatively large amounts of androgens. This confirms the report of Riley and Hammond (8).

Effect of lactation upon androgen excretion. The data on the individual cows were tabulated according to the stage of lactation (table 3). It will be seen that no trend in the average comb weight with the advance of lactation is present during the first 8 months. Comb weight values above normal in the ninth and eleventh months of lactation are interpreted as indications of the prepartum rise in androgen excretion rather than relationship to the advance in the period of lactation.

The tabulation of the data by breeds, up to the time of the preparturient rise, again indicated little difference in the androgen excretion by Holstein and Guernsey cows. The Jersey cows, however, again were higher but not quite as high as in the tabulation of pregnant animals.

DISCUSSION

While the data are limited, they indicate that a relatively high average level of androgen excretion occurs in unbred heifers and non-pregnant, non-lactating cows. Since this observation is in agreement with that of Riley and Hammond (8), it would appear that this hormone is excreted at relatively high levels in sexually mature heifers without reference to pregnancy. Whether there is a cyclic variation in the androgens in relation to the period of heat in heifers has not been investigated. Since there is much experimental work indicating that androgens can stimulate the growth of the mammary duct system, the cyclic growth of the pubertal duct system of heifers may be due, in part, to the presence of androgens as well as estrogens in the blood at this time.

Following conception, the rate of androgen excretion is not believed to increase markedly. It is true that these data show a high level during the second month of pregnancy with a reduction until the seventh month. Further data

TABLE 3
Effect of the stage of lactation upon the androgen excretion of dairy cattle (assay with white rock chicks—28 days of age)

Month of lactation	Stage of preg- nancy av.	No. of cows (breeds)	Female Chicks				Male Chicks				Male + female 2	
			No. of chicks	Av. body weight (g.)	Av. testes weight (mg.)	Av. comb weight (mg.)	Comb weight 100 g. body wt.	No. of chicks	Av. body weight (g.)	Av. ovary weight (mg.)		Av. comb weight 100 g. body weight (mg.)
1	open	8 (6H, 1G, 1J)	86	263.2	33.48	205.85	78.21	72	234.6	44.21	127.46	54.33
2	open	8 (3H, 1G, 4J)	73	238.2	30.36	151.05	63.41	70	230.8	46.40	93.05	40.32
3	{ 3 open 2 bred	5 (4H, 1J)	52	261.7	32.56	206.27	78.92	51	251.1	47.25	128.58	51.21
4	{ 3 open 2-29 days	6 (3H, 1G, 2J)	54	262.5	30.55	205.68	78.35	62	234.2	37.05	149.69	63.92
5	{ 4 open 2-31 days	6 (5H, 1G)	74	256.1	36.87	204.55	79.87	50	238.8	47.73	133.99	56.11
6	{ 1 open 5-67 days	6 (4H, 1G, 1J)	61	249.6	31.86	206.65	82.79	58	220.5	49.40	134.94	61.20
7	{ 2 open 2-76 days	4 (4H)	33	269.3	40.75	183.79	68.25	45	239.3	41.58	117.17	48.96
8	116	5 (4H, 1G, 1J)	59	257.1	35.76	179.51	69.82	58	234.8	50.93	93.19	39.69
9	137	7 (4H, 1G, 2J)	70	233.3	28.92	215.99	92.58	67	235.1	39.98	173.83	73.94
10	164	3 (1H, 1G, 1J)	31	267.3	36.03	178.73	66.86	30	244.4	43.26	127.50	52.17
11	217	4 (3H, 1G)	44	256.3	28.10	324.40	126.41	30	232.0	34.68	286.09	123.30
Breed												
Jersey	all	33	374	251.6	34.62	184.49	73.33	364	236.1	50.68	126.34	53.51
Holstein	all	6	93	269.3	34.67	200.47	74.44	76	247.7	44.17	110.01	44.41
Guernsey	all	10	167	255.3	28.93	212.65	83.29	166	234.9	41.44	139.72	59.46

* H = Holstein, G = Guernsey and J = Jersey

will be required to indicate whether the rise during the second month is significant. Until that time, it seems preferable to believe that early pregnancy is not a period of increased androgen excretion.

It is well known that the first half to two-thirds of pregnancy is a period of rapid duct and lobule-alveolar growth of the udder. This growth is stimulated by the hormone of the corpus luteum, called progesterone, acting upon the anterior pituitary thus stimulating the secretion of the mammogenic hormone. It has been proved that the estrogenic hormones augment the action of progesterone and mammogen. It also has been shown that certain androgenic hormone derivatives can stimulate slight lobule-alveolar mammary growth (7). The fact that the androgenic hormones are not excreted in increased amounts during the first two-thirds of pregnancy suggests that they do not play a predominant role in the great growth of the udder at this time. They may supplement the progesterone and balance physiologically the increasing secretion of estrogen.

The most striking change in the androgen excretion rate occurs during the period preceding parturition. It is well known that the excretion of estrogen both in the urine (15) and feces (2) increases rapidly at the approach of calving in dairy cattle. It is possible that the rise in androgen excretion at this time indicates a mechanism designed to counter-balance or offset, in part, the physiological effect of the rapidly rising prepartum estrogen secretion. It is believed that the secretion of progesterone may decline at this time, thus permitting estrogen to become predominant and to initiate parturition and, by stimulation of the pituitary, to increase the secretion of the lactogenic hormone (5, 6).

Since estrogen has been shown to stimulate the secretion of adrenocorticotrophic hormone by the pituitary, there would be expected increased gluconeogenesis of protein and resultant loss of nitrogen in the urine due to the hormones of the adrenals (3, 9). The androgens are known to have the opposite effect, increasing the retention of nitrogen and body growth by reduction of the secretion of the adrenal cortical hormones (16).

The concurrent rise in both estrogen and androgen during late pregnancy may indicate the presence of an adaptive mechanism of the body by which certain effects of one hormone can be balanced by the opposite effects of the other yet permitting necessary stimulation to prevail, *i.e.*, the estrogen stimulation of the lactogenic hormone.

The rise in androgen secretion at the approach of parturition suggests the need of further study of the relation of estrogen to androgen in the stimulation of the lactogenic hormone. It has been shown that androgenic hormones are capable of stimulating an increase in the lactogenic hormone of the pituitary (5). Does the androgen secreted prepartum supplement estrogen in the stimulation of the lactogenic hormone?

Since the level of excretion of androgenic hormones during most of lactation is rather uniform, there is no reason to believe that the androgens play a dynamic role in the maintenance of milk secretion.

SUMMARY AND CONCLUSIONS

1. Manure from cows of the Guernsey, Holstein and Jersey breeds during various physiological states has been dried at 45° C. and assayed biologically for its content of male (androgenic) hormone.

2. It was observed that sexually mature non-pregnant heifers excrete male hormone at a level comparable to those of mature cows.

3. During the first two-thirds of pregnancy, no tendency for a rise in androgen excretion was observed. There was evidence of a preparturient rise in androgens.

4. With the advance of lactation, no change in androgen excretion was noted except when associated with the approach of the subsequent parturition.

5. Dried cow manure from the Guernsey and Holstein breeds appeared comparable in biological activity; the Jersey cows appeared to excrete slightly more male hormone.

6. It is suggested that the preparturient rise in androgen may be related to the marked rise in estrogen at the same time.

7. It is possible that androgens as well as estrogens play roles in the stimulation of the secretion of the lactogenic hormone by the pituitary at the time of parturition.

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THE INFLUENCE OF THE RATION AND RUMEN INOCULATION ON THE ESTABLISHMENT OF CERTAIN MICROORGANISMS IN THE RUMENS OF YOUNG CALVES¹

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INTRODUCTION

Previous investigations concerning the etiology of digestive disturbances in young calves included a study of the microorganisms which appeared in their rumino-reticular cavities (7). It was observed at that time that rumen fauna and certain characteristic flora similar to those seen in samples from mature animals were not established in the majority of the calves examined until they were several weeks old. Upon direct inoculation of organisms from cows into the rumens of a few calves, the organisms became established in some of them. These calves, which were on various rations, were among those in the herd at that time which progressed most satisfactorily, but proper controls were not included. The investigations were continued for the purpose of attempting to determine if there was any material advantage in stimulating the development in calves of early rumen activity comparable to mature animals.

Limited studies with a few young calves indicated that certain microorganisms characteristic of the rumen flora and fauna failed to become established regardless of how often inoculations were made when most of the dry feed ingested was grain. It generally was possible, on the other hand, to establish these particular rumen microorganisms in calves even before they were a week old, provided they were ingesting good quality hay and no grain. Variations in rumen flora which were related to the feed ingested have been reported for sheep by Elsdon (4), who also cited van der Wath's findings on the same subject. Phillipson (6) also makes reference to this variability of the flora associated with ration differences. It would be expected that a similar situation would exist as regards young calves.

As a result of the preliminary investigations, it was decided to place 4-day-old calves on various systems of feeding, both with and without rumen inoculations, in order to study further the significance of the previous observations. Clinical studies and repeated examinations of the rumen flora and fauna were carried out on these calves during their first 6 weeks of age. Blood plasma vitamin A, carotene and ascorbic acid determinations were made at frequent intervals on many of the calves used in this experiment. The results are reported elsewhere (5).

METHODS

Young calves of both the Jersey and Holstein breeds which had received colostrum usually for 3 days were placed on twice-a-day pail feeding of pasteur-

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ized milk between their second and fourth days of age. They were fed at the daily rate of approximately 0.9 lb. for each 10 lb. of body weight at birth, but within the limits of a minimum of 5 lb. and a maximum of 10 lb. per day. It was hoped to encourage the consumption of dry feeds at an early age by limiting milk consumption to this comparatively low amount. They were treated variously as regards the dry feeds given them. Calves which received hay had access to it while with their dams or were provided on their first day of age with 1 lb. of green alfalfa hay which occasionally had some grasses mixed in it. This was replenished or replaced frequently so that the calves had fresh hay before them at all times. Grain, when included in the ration, was fed once per day as long as the quantities did not exceed 0.5 lb. per day. It consisted of a 14 per cent protein herd mixture of corn, oats, wheat bran and soybean oil meal.

Rumen inoculations were given to some of the calves by passing pieces of freshly obtained cuds from cows into the posterior of the calves' mouths. The cows were being fed alfalfa hay, grain and silage. The inoculations generally were carried out on the fifth, tenth, fifteenth and twenty-first days of age. Samples were obtained from the fore-stomachs of the calves with stomach tubes, using the syphoning method developed for use with the Colorado rumen lavage tube, and, in some instances, samples were taken at the time of slaughter. No difference as regards flora and fauna was observed in samples obtained from the same calves by the two methods.

Calves which did not receive rumen inoculations were cared for by the same personnel as the others and were housed in the same buildings. However, they were separated from direct contact with the other calves and mature animals by partitions and passageways. A limited effort was made to avoid transferring organisms from one calf to another on milk buckets and other equipment.

The rumen samples were examined under the microscope in the fresh condition, using slides and cover glasses, for the purpose of observing the protozoa. For the most part, Gram stained smears were relied upon for bacteriological purposes. In preparing the slides, some of the thick soupy materials containing particles of feed were included, since Baker (2) has shown that some varieties of bacteria tend to remain rather closely attached to feed particles and may not always be readily visible in the free liquid. Descriptions of microorganisms based on morphological and staining characteristics leave much to be desired. However, this method was considered the most advantageous to use for evaluating rumen bacteriological activity under the conditions of this investigation.

RESULTS

The Microorganisms

The protozoa encountered in the rumen samples from the calves appeared to be similar to those mentioned by others, including several investigators whose observations were cited by Baker and Harriss (3) in their recent review article. All those commonly seen in samples from cows were established readily upon

inoculation into calves which were ingesting suitable feeds including hay and hay-plus-grain rations.

Many bacteria differing in morphology and staining characteristics were visible in the rumen samples. Many of them have been described previously by others including Baker (1, 2) and Baker and Harriiss (3). Some varieties of organisms were observed to be noticeably present only when appreciable quantities of hay were being consumed and certain other varieties when grain was the principal dry feed ingested. This does not mean that these particular organisms alone were present under such conditions, but merely that they at least were readily visible in the smears. However, when the proportion of grain consumed was high, a few varieties of organisms including those described herein sometimes would appear to make up the majority of the bacterial population. Proof neither was sought nor obtained that the organisms mentioned were the most important ones in the digestion of the feeds present. They were used in this study

TABLE 1

Classification and description of some calf rumen flora observed to vary with the type of feed eaten

Hay Flora*	
Group I	Quite large G+ coccoids in closely knit pairs
Group II	Large G+, thick, fairly square-ended rods
	Very large G- cigar-shaped rods
	Smaller G- short rods in fours and multiples of 4
Grain Flora*	
Group I	Medium-sized, comparatively thin, G+ rods (sometimes granular stain and variable length)
Group II	G- rods resembling coliform.

* Flora which appeared to be characteristically associated with the ingestion of these feeds.

as indicators of the presence or absence of characteristic bacterial populations. As reported elsewhere, further studies showed that the same organisms possibly might be used as indicators, within certain limits, of the relative ratios of grain and hay being ingested by young calves (8).

It was possible to subdivide the varieties of the organisms which were noticed to be associated with hay consumption into two sub-groups (table 1). The first consisted of large Gram-positive coccus organisms in closely knit pairs and sometimes in groups of four or more, but not in chain formations. The approximate size of the pairs was 2.8×2.3 microns, and some groups composed of more than a single pair were as much as 4.4×4.0 microns. They possibly were similar to those called large sarcina packets by Baker (1). Among the organisms included in the second hay sub-group were large Gram-positive, thick, rather square-ended rods whose length varied between 3.2 and 5.5 microns and which were approximately 2.5 microns wide. They were observed quite frequently in pairs. Gram-negative, extremely large, cigar-shaped organisms, which often were as much as 21.5 microns long and approximately 4.0 microns wide, also were included in this sub-group. It is probable that these latter organisms are similar to those referred to by others as giant elipsoidal forms (3) or *Ocillospira* (1, 2). Besides

these, in this group were Gram-negative rods of approximately 1.0×0.8 microns in size that tended to group in fours and multiples of four in shapes suggestive of window panes.

Organisms associated with grain consumption also could be sub-divided into two groups (table 1). The first consisted of Gram-positive rods which ranged between 1.7 and 3.4 microns in length and were approximately 0.8 microns in width. They appeared to resemble lactobacilli (10). Present in some smears were masses of either short varieties of this organism or a different organism of similar Gram staining property. Sometimes, especially when considerable numbers were present, a tendency existed for these organisms to stain in a granular manner. The second sub-group were Gram-negative and morphologically resembled coliform organisms or did not differ much from them.

Photomicrographs of the various organism types are reproduced in figure 1.

Variations in the establishment of microorganisms

Calf group 1 (hay plus rumen inoculation). Protozoa and bacteria of the two groups noted to be associated with hay ingestion were established in the ru-

TABLE 2

The influence of the ration and rumen inoculation on the establishment of certain micro-organisms in calf rumens

Calf group	Ration	Age	No. calves examined	No. calves protozoa present	No. calves hay flora present		No. calves grain flora present	
					Group I	Group II	Group I	Group II
		(wks.)						
I	Hay plus inoculation	3	8	8	8	8	0	0
		6	7	7	7	7	0	0
II	Hay, uninoculated	3	8	0	8	3	0	0
		6	6	0	6	5	0	0
III	Hay and grain plus inoculation	3	7	6	2	1	2	0
		6	7	7	6	2	4	0
IV	Hay and grain, uninoculated	3	5	1	2	2	4	1
		6	5	1	2	2	5	3
V	Calf starter, uninoculated	3	4*	0	0	0	4	4
		6	2*	0	0	0	2	1

* 2 Calves received hay.

mens of eight calves, which had good quality alfalfa hay available to them from birth, before they reached the age of 3 weeks. The organisms still were present several weeks later in the seven calves which were continued on the experiment. "Grain-type" organisms were extremely scarce in samples from these calves (table 2).

Calf group 2 (hay without rumen inoculation). Protozoa failed to make their appearance in samples from eight similarly-fed but uninoculated calves up to the time they reached the age of 3 weeks and up to 6 weeks in the case of the six of them that were continued on experiment. "Hay flora" of the paired coc-

cus type developed in all eight calves by the time they were 3 weeks old and continued to be present in samples from these calves throughout the experimental period. Samples from all but three of the eight calves contained organisms of the second hay sub-group when the calves were 3 weeks old. By 6 weeks of age, samples from five of the six calves contained some of at least one of the organisms of this sub-group. Organisms of the two grain types also were extremely scarce in samples from these calves (table 2). A fairly large (Gram-positive rod, thicker and more tapered at the ends than the one seen in association with grain feeding, was observed in great numbers in samples from two of these calves by the time they were 3 weeks old and in five of the six at 6 weeks of age. These organisms never were observed in appreciable numbers in samples from any of the other calves.

Calf group 3 (hay, grain, plus rumen inoculation). Seven inoculated calves were fed good quality alfalfa hay from birth and a simple 14 per cent protein grain mixture starting on the 14th day of age, both free choice. Protozoa became established in six, hay flora of the paired coccus organism sub-group in two, and hay flora of the second hay sub-group in one, by the time the calves were 3 weeks of age. By 6 weeks of age, samples from all seven had protozoa present, six contained hay flora of the first sub-group and two of the second. Gram-positive grain-type flora developed in two calves by 3 weeks and in four calves by 6 weeks of age (table 2).

Calf group 4 (hay plus grain without rumen inoculation). Only one of five uninoculated calves on a schedule of hay and grain similar to group III developed rumen protozoa by 4 weeks of age or even by 6 weeks of age. Flora of both hay groups were present in samples from two of the five calves by the time they were 3 weeks old but had not yet appeared in the other calves at 6 weeks of age. Bacteria of the first grain sub-group were visible in samples from four calves at 3 weeks of age and in all five calves at 6 weeks of age. Gram-negative bacteria of the second grain sub-group were observed in samples from one calf at 3 weeks and from three calves at 6 weeks of age (table 2).

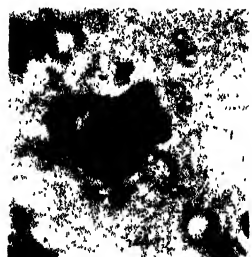
Calf group 5 (calf starter grain ration without inoculation). Four uninoculated calves were fed a commercial calf starter grain ration in pellet form. Two of them received good quality alfalfa hay in addition. Both feeds were given free choice starting on the fourth day of age. The amount of milk fed one of the two calves receiving hay gradually was reduced as the calf increased its consumption of the grain. At 3 weeks of age, no protozoa or hay-type bacteria could be seen in samples from any of these four calves. However, great numbers of the grain-type organisms were present almost to the exclusion of all other bacteria. Only the two calves receiving hay were continued on experiment beyond 3 weeks of age. A similar condition was noted in them when they were 6 weeks of age, although the Gram-negative bacteria resembling coliform organisms appeared to be less prevalent (table 2).

Samples from approximately 20 calves of similar age and on rations fairly similar to those used for the last two groups had been examined repeatedly the previous year. The results were very much the same.

Calf group 6 (shavings and straw bedding without inoculation). Neither protozoa nor hay-type flora were established in any one of seven uninoculated calves which were fed milk alone and bedded with shavings or straw up to the time they were 3 weeks of age. It also was questionable whether any of the



Hay Group I Organisms
(pairs and multiples of pairs)



Hay Group II Organisms
(thick rod, cigar-shaped rod, and group of small rods)



Grain Group I Organisms
(medium-sized rods)

FIG. 1. Photomicrographs of calf rumen microorganisms
(approximately $\times 1800$)

grain-type flora were present in the samples. In fact, relatively few organisms of any kind were present even though all calves ate some of the bedding. Medium-sized, spindle-shaped bacteria which often contained spore-like bodies were present in appreciable numbers in samples from five out of six of these calves

which were bedded with shavings. These were the only ones in which this organism was ever observed.

Growth of the calves. An effort was made to have the two breeds about equally represented in each group with the exception of group VI, which was composed entirely of Jerseys. The average gains in weight made by the calves in all six groups were between 7 and 9 lb. per calf for the first 3 weeks. The calves in groups I, II, III and IV made an average gain of 15 to 16 lb. per calf during the second 3 weeks, while those in group V gained an average of 22 lb. Group VI was discontinued at the end of the first 3 weeks. These gains are lower than the standards given for calves of comparable breeds by Ragsdale (9). Although gain in weight was desired, it was not an objective in these experiments.

Health of the calves. The hair coats of calves on hay alone without rumen inoculation (Group II) appeared to be rougher than those on similar feed which received the inoculation (Group I). Any difference resulting from inoculation was less noticeable or non-existent between the groups of calves receiving rations containing grain. As reported elsewhere (5), calves in group I receiving hay and inoculation maintained on the average uniformly higher blood plasma levels of ascorbic acid during their first 6 weeks of age than any other group. The clinical manifestations of sickness were limited to digestive tract upsets. No trouble was experienced in this respect among the calves in groups I and II, although two calves in each group had rather soft feces on a single day each. The incidence of diarrhea among the calves in group III was 57 per cent, group IV 66 per cent, group V 75 per cent and for group VI 70 per cent. The duration of individual attacks ranged between 2 and 8 days.

Sources of the organisms other than from rumen materials. The organisms designated as Hay Group I and those associated with grain established themselves more readily by natural means in the young calves than did the protozoa and the organisms in Hay Group II. This indicates greater availability of sources of the former. Feces would be a logical source of organisms, augmenting the organisms spread through slobbers from cud-chewing mature animals. Baker (2) examined the feces from a bovine fed on hay and various concentrate rations for some of the characteristic organisms. He concluded that the majority of these organisms were destroyed on passage through the intestines. This seemed to be true based on our Gram stain examinations of fecal samples. However, limited numbers of organisms resembling those designated as Hay Group I and also the Gram-positive varieties of those associated with grain ingestion were present in seven fecal samples from rumen inoculated calves on hay and milk alone. They apparently also were present to a lesser extent in two of four samples from similar calves eating both hay and grain, and in six out of seven samples from cows on mixed rations. Although the varieties associated with grain ingestion were visible in three samples from calves on calf starter ration, no hay-type bacteria could be observed. A young calf was given, by stomach tube, repeated rumen inoculations with feces from a 5-month-old inoculated calf on a hay and skim-milk diet. None of the second-hay-group bacteria or rumen protozoa became established in its rumen even though the first-hay-group organisms did so. Thus,

it would appear that feces from older stock may provide a source from which some of the rumen microflora may be obtained by young calves.

DISCUSSION

It would appear from the present observations that rumen protozoa encounter difficulty in being transferred to young calves under conditions which frequently exist on dairy farms. However, their importance as regards the well-being of the host animals has not been fully established. It is not possible from our data to deduct that they were responsible for the higher blood plasma ascorbic acid levels reported elsewhere (5), or the better appearance of the inoculated calves which received hay alone. In fact, there is more indication that flora were involved because the calves in group I maintained higher blood plasma levels of ascorbic acid than group III, yet both had protozoa in their rumens. Furthermore, a much less satisfactory condition as regards hay-type flora existed in group III as compared to group I.

The type of feed ingested appeared to be the principal factor which influenced the establishment of the organisms designated as group I of those noted to associate with hay and both groups of organisms observed in association with grain ingestion. Evidently the same was partly true of organisms designated as group II of those observed to associate with hay, although the figures indicate a less satisfactory source of organisms for inoculation of the calves than existed for the former. The failure of inoculation to establish hay varieties of flora in calves provided with both hay and grain was unexpected and difficult to explain until later experiments were conducted. These showed that once the ratio of grain ingested exceeded the hay, the proportion of hay varieties of flora appeared to markedly decrease in the Gram stain preparations of rumen samples (8). Thus, it appears that the logical explanation is that some of the young calves tended to eat proportionately more grain than hay when both were offered free choice.

The practical observation that the early development of mature rumen function in young calves may be influenced for the better by inoculation, under some conditions, is probably of some significance. Whether the microscopic observations as outlined here are sufficiently sound and adequately described must await further experimental work under varied conditions. The varieties described as being associated with hay ingestion are sufficiently characteristic in morphology that their recognition probably is quite reliable. However, because of the lack of definite morphological individuality, recognition of organisms designated as associated with grain ingestion possibly is less accurate.

Quite probably the rumen flora may vary somewhat between herds. A slight indication of this has been obtained from examinations conducted on calves in a few other herds and from the reports of others. However, fairly similar conditions probably exist in the majority of herds as regards rumen microorganisms, and observations made on calves in various locations may be comparable.

The comparatively low milk consumption of the calves used in these experiments probably was responsible, especially during the first 3 weeks of age, for

the fact that weight gains were lower than accepted standards (9). The total consumption of milk during the 6-week-period was as low as 210 lb. each for most of the Jersey calves and only two Holsteins received more than 336 lb.

Apparently, rumen inoculation did not influence the ability of the calves to withstand the factors which existed in the herd that stimulated attacks of diarrhea. On the other hand, the type of ration fed, especially good alfalfa hay and milk, appeared to be of more value in preventing the occurrence of this malady. This naturally raises the question as to whether or not, under some conditions, the health of the digestive tracts of young calves may be jeopardized as the result of attempts to make rapid gains in weight at very early ages.

SUMMARY

The rumens of young calves being fed milk and various dry feed rations were inoculated with microorganisms from the rumens of mature stock by placing pieces of cuds from the latter in the posterior of the mouths of the calves. The inoculations were omitted from similarly fed calves used as controls.

The inoculations assisted in the establishment of protozoa in the rumens of calves eating either hay alone or both hay and grain. They assisted in the establishment of some, but not all, of the characteristic varieties of rumen microflora which were associated with hay ingestion in calves fed on alfalfa hay alone. The establishment of varieties of organisms which were associated with the ingestion of grain was not assisted by the inoculations. The establishment of the varieties of flora which were associated with hay ingestion was inhibited in some calves when grain was fed.

The inoculated calves on a diet of alfalfa hay and milk alone were considered to have a better appearance than the controls, but this difference was not apparent between the inoculated and uninoculated groups fed on both hay and grain. Data reported elsewhere (5) show that uniformly higher levels of ascorbic acid in the blood plasma were maintained during the first 6 weeks following birth in the inoculated calves fed alfalfa hay and milk alone than in the calves of any other group. Gains in weight by the calves were very similar in all groups during the first 3 weeks of age. During the second 3-week period, all groups made similar gains except group V, which received a commercial calf starter grain ration. The two groups of calves fed on alfalfa hay and milk alone were free of diarrhea, but the incidence in all other groups was in excess of 50 per cent.

Feces were examined in a search for sources of organisms which resembled natural inhabitants of rumens and some appeared to be present.

The authors wish to acknowledge the assistance of Mr. John Tate, Mr. R. L. Johnson, and Mr. C. E. Knoop in conducting this investigation.

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THE INFLUENCE OF THE RATIO OF GRAIN TO HAY IN THE RATION OF DAIRY CALVES ON CERTAIN RUMEN MICROORGANISMS¹

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Variations were observed to occur in certain flora and fauna present in rumen samples from young calves which apparently depended upon the ratio of grain to hay that the calves ingested (1). This indication was pursued further to determine if the observed variations were consistent. Should this be the case, it was hoped that examinations of rumen contents and rumen microorganisms might prove to be more valuable in making differential diagnoses of some calf problems. For instance, information of what feeds have been ingested often is essential in order to determine with any degree of accuracy if a relationship exists between the feed and the unhealthy condition of the calves. Yet, it often is difficult to estimate what the calves actually have been eating when more than one feed has been offered free choice, including the bedding. This problem is complicated further when several calves are fed together in groups because of the individual variations that exist between calves in their choice of feeds.

It perhaps is of interest to add here that extreme variations in rumen microfauna and microflora were observed to be more frequent among young calves fed dry feeds free choice during their first few weeks of age than among similarly-fed older calves. This situation probably resulted from the fact that younger calves ate limited quantities of feed and often limited themselves to only one feed at a time. Because of the relatively small capacity of their rumens, the influence of eating a single feed on the microorganisms was much greater than in older calves in which the buffering effect of larger amounts of previously-eaten feeds existed.

METHODS

The 19 calves used were between 1 and 4.5 months of age. Rumen flora and fauna, which had been classified as quite characteristic, had been present in all the calves prior to the time this particular study was undertaken. Most of them had been inoculated by use of cuds from mature animals in the manner outlined previously (1). It was difficult to determine accurately the relative quantities of hay and grain ingested by calves younger than these and, consequently, data from such calves were omitted.

The quantities of hay and grain consumed during the 4 days prior to the examinations were used in arriving at the relative ratios of the two feeds eaten by the calves. However, they actually were consuming approximately the same proportions for some days longer than this. This period was chosen on the basis of experience with the establishment of characteristic flora and fauna in the rumens of young calves.

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Some calves received different ratios of hay to grain at different times either by design or their own choice and, consequently, data from them were included in more than one category. The hay was fairly good quality alfalfa hay and the grain was a simple 14 per cent protein mixture containing 4 parts of corn, 3 of oats, 1 of bran and 1 of soybean oil meal.

Rumen samples were obtained by use of stomach tubes and examined microscopically in the fresh state and by use of Gram stains as outlined previously (1). The preparations were examined for the same organisms as those discussed in the previous paper (1). Two main groups of bacteria were noted, one associated with hay ingestion and the other with grain, and two sub-groups were recognized in each group.

RESULTS

The results of 35 examinations are summarized in table 1. Column 3 in the

TABLE 1
The influence of the ratio of grain to hay on some calf rumen microorganisms

Ratio Grain : Hay	No. rumen samples	No. samples protozoa present	No. samples Hay flora present		No. samples Grain flora present	
			Group I	Group II	Group I	Group II
0 : 1	19	19	19	19		
1 : 3	6	6 ^b	6	6	1	1
2 : 3	4	3 ^b	4	2	4 ^a	2
1 : 1	6	6 ^b	6	3	3	5
3 : 2	3	2 ^b	3	2		
3 : 1	2	2 ^a			1 ^b	1 ^b
1 : 0	5	1 ^a			1	1
					4 ^b	3 ^b
						1

^a = few; ^b = masses; unmarked = moderate or appreciable numbers.

table shows that protozoa were present in all samples except four of the five from animals on grain without hay. Moderate numbers were observed to be present when the dry feed ration consisted of hay alone. With the addition of some grain to the ration, the numbers of protozoa in the samples increased greatly. This is in agreement with the findings of others as summarized by Phillipson (2). A marked reduction in numbers followed, once the ratio exceeded 3 parts of grain to 2 parts of hay. Limited numbers only were present in samples from calves eating three or more times as much grain as hay. The one calf receiving grain without hay, but still having protozoa present in the rumen, was one of the oldest calves used. It had been fed grain with straw for bedding for 10 days at the time of the examination.

Organisms which were classified as belonging to the hay flora groups were visible in Gram stain preparations of all 19 samples from calves on rations of hay alone. The prevalence of these organisms in the smears appeared to in-

crease with the addition of some grain to this ration. However, as the proportion of grain ingested approached quantities equal to the hay, a reduction was rather apparent. Organisms of the second hay group were reduced more noticeably than those of the first hay group in samples from animals eating as much or more grain than hay. As shown in columns 4 and 5, both groups of the organisms associated with hay were missing from the samples once the ratio reached 3 parts of grain to 1 of hay. Thus, the organisms associated with hay consumption disappeared from the rumen samples at lower ratios of grain to hay than did the protozoa. The apparent increases in the flora associated with hay ingestion on the addition of some grain to rations of hay alone may have resulted from the eating of more balanced rations by the calves. Such is suggested by the observations of Van der Wath, as cited by Phillipson (2), that bacterial numbers were influenced by the diet, with balanced rations being the most satisfactory.

Only limited numbers of the bacteria which were observed to associate with grain rations were visible in the samples until a proportion of 3 parts of grain to 2 of hay was being consumed (columns 6 and 7). These organisms increased in relative prevalence in comparison with other flora as the proportion of grain increased. On rations of grain alone, some samples appeared to contain practically no other organisms.

Very small Gram-negative organisms were noticeably prevalent in samples from calves on rations containing hay alone or high proportions of hay. Small Gram-positive short rods or cocci were observed in increasing proportions on the addition of grain to rations of hay.

Although data collected on this group of calves are very limited, they indicate that by observing certain flora and fauna present in rumen samples from calves, it may be possible to estimate the relative ratio of grain to hay that they are ingesting.

SUMMARY

A total of 35 rumen samples from 19 calves between the ages of 1 and 4.5 months were examined microscopically. The calves received rations of alfalfa hay or grain alone, or various proportions of these. Most of them had received rumen inoculations and the remainder had been exposed to usual rumen micro-organisms in a natural manner.

Moderate numbers of protozoa and flora of varieties observed to associate with hay ingestion accompanied the ingestion of hay without grain.

Masses of protozoa along with fairly numerous flora of the 2 hay groups were associated with the consumption of hay and moderate quantities of grain.

Similar concentrations of protozoa, accompanied by rather limited numbers of organisms of the hay groups and fairly numerous bacteria of the varieties observed to associate with grain consumption, accompanied the ingestion of approximately equal quantities of hay and grain.

Limited numbers of protozoa accompanied by great numbers of bacteria of the grain groups, but no organisms of the hay groups, were present when the ration consisted of almost all grain.

Protozoa and organisms of the varieties associated with hay ingestion generally were absent entirely in samples from calves on strictly grain rations.

The authors wish to acknowledge the assistance of Mr. John Tate, Mr. R. L. Johnson and Mr. C. E. Knoop in conducting this investigation.

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THE INFLUENCE OF THE RATION AND EARLY RUMEN DEVELOPMENT ON THE CHANGES IN THE PLASMA CAROTENOIDS, VITAMIN A AND ASCORBIC ACID OF YOUNG DAIRY CALVES¹

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The period between birth and the time a calf has developed into a normally-functioning ruminant is recognized as critical from the standpoint of nutritional well-being and health. As pointed out by Pounden and Hibbs (8), this period may extend for many weeks in some cases, when judged by the presence or absence of characteristic rumen microorganisms.

Several reports have appeared in the literature showing the usual changes in the blood plasma carotenoids and vitamin A of calves from birth to several weeks of age (1, 2, 3, 6, 11, 12, 14, 15). The changes in plasma ascorbic acid have been reported by Phillips *et al.* (7), Hibbs and Krauss (2), Sutton and Kaeser (12), and Teeri *et al.* (14).

Wise *et al.* (15) have reported results showing that after the blood carotenoids reach a peak on about the third day, as the result of colostrum feeding, there is a rapid decline for from 4 to 5 weeks and then a gradual rise to the post-colostrum feeding level or slightly above at 8 to 10 weeks of age. Vitamin A follows a somewhat similar trend. Teeri *et al.* (14) report that, on the average, blood carotenoid values level off between 15 and 23 weeks of age at about 44 γ per 100 ml. in Holstein calves and at about 65 γ per 100 ml. in Jersey and Guernsey calves. Considerable individual variation is indicated by the high and low values obtained in calves apparently fed and managed alike.

It is striking that until most calves are several months old their blood carotenoid levels do not even approach the levels found in mature animals fed on dry feeds. This may be the result of the ability of the mature animal to consume relatively large quantities of roughage. It is not illogical, however, in the light of our previous observations regarding the variations in the rate of establishment of characteristic rumen microorganisms in calves (8), to assume that the differences in the blood carotenoids and vitamin A levels between calves and adult animals might be due, at least in part, to their relative ability to digest roughage in the rumen. Furthermore, many of the individual variations found among calves may be due, in part, to the differences in the age at which normal rumen function begins.

Investigations were undertaken, therefore, to study the influence of the ration and rumen inoculations on the establishment of rumen function in young calves, the results of which are reported elsewhere (9, 10). Concurrently, a study was made on the influence of the ration and early rumen development on the changes

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occurring in the plasma carotenoids, vitamin A and ascorbic acid of dairy calves during their early postnatal development.

EXPERIMENTAL

Plasma vitamin A and carotenoids were determined by the method described by Kimble (4). The method of Mindlin and Butler (5) was used to determine plasma ascorbic acid.

After a colostrum feeding period, usually 3 days, all calves were fed whole milk for the entire experimental period of 6 weeks at the rate of 0.9 lb. per 10 lb. of body weight at birth. This relatively low level of milk feeding was adopted in order to encourage the calves to consume more of the dry feeds offered.

Beginning on the fourth day of age, calves born in the herd from January until April, 1948 were assigned to one of six groups and fed whole milk and various dry feeds with and without rumen inoculations as follows: Group I, alfalfa hay plus rumen inoculations; Group II, alfalfa hay alone; Group III, alfalfa hay plus grain (14 per cent protein herd ration) plus rumen inoculations; Group IV, alfalfa hay plus grain (14 per cent protein herd ration); Group V, alfalfa hay plus standard calf starter pellets; Group VI, whole milk only (this group was continued on experiment for only 3 weeks).

The rumen inoculations in groups I and III were accomplished by direct transfer of small pieces of cud material to the mouths of the calves from cows in the herd. This was done in order to make certain that these calves had access to the microorganisms normally present in the rumens of the adult animals.

All calves were bled as nearly as possible on the fourth and seventh days of age and weekly thereafter until the forty-second day of age and the plasma carotenoids, vitamin A and ascorbic acid were determined.

The results of the blood analyses are shown in table 1. No beneficial effect of rumen inoculations on the blood plasma carotenoids was observed. Average figures for the calves in groups III, IV and V, which were fed grain, show that when grain was included in the ration, plasma carotenoids did not increase during the first 6 weeks to the extent observed in groups I and II, which were fed alfalfa hay as the only dry feed. The calves in groups I and II made an average steady increase in plasma carotenoids from 4 days until 42 days of age, reaching extremely high levels as compared to any of the other groups.

Plasma vitamin A was found to decrease markedly from the fourth until the forty-second day of age in all groups except group V, which received a calf starter containing 5,000 U.S.P. units of supplemental vitamin A per lb. No marked beneficial effect from rumen inoculations was noted on the plasma vitamin A level although the values for vitamin A appear to be somewhat higher in group III as compared to group IV.

The level of plasma ascorbic acid was found to decline between the seventh and fourteenth days of age in all groups except group I, which was fed alfalfa hay plus rumen inoculations. This group maintained the most uniformly high level of plasma ascorbic acid during the first 4 weeks of any of the groups.

TABLE 1
The influence of the ration on the changes in the plasma carotenoids, vitamin A and ascorbic acid of the blood of young calves

Group ^a	No. of calves	Plasma carotenoids Ages of animals						
		4 days	7 days	14 days	21 days	28 days	35 days	42 days
		Plasma carotenoids (γ/100 ml.)						
I	6	32.3 ± 6.5 ^b	34.9 ± 5.5	42.5 ± 4.4	58.2 ± 16.3	76.9 ± 14.2	93.2 ± 20.0	99.2 ± 23.3
II	6	35.9 ± 5.7	38.2 ± 5.8	49.9 ± 14.0	53.6 ± 19.4	67.9 ± 23.9	92.6 ± 20.0	96.6 ± 22.0
III	7	20.6 ± 2.9	20.7 ± 3.1	34.0 ± 7.7	37.0 ± 12.7	32.7 ± 9.1	36.2 ± 10.7	49.7 ± 3.3
IV	5	21.5 ± 3.9	32.7 ± 9.8	36.2 ± 7.5	35.4 ± 13.7	44.4 ± 12.3	44.4 ± 12.3	56.8 ± 15.4
V	4	38.4 ± 20.0	32.7 ± 8.4	35.1 ± 8.9	31.4 ± 10.3	33.0 ± 0.0	35.5 ± 10.7	28.3 ± 2.6
VI	6	19.4 ± 3.5	22.4 ± 4.5	27.5 ± 4.6	20.0 ± 2.6			
Plasma Vitamin A (γ/100 ml.)								
I	6	20.3 ± 3.3	16.4 ± 3.1	14.8 ± 1.5	12.1 ± 0.9	10.8 ± 1.2	9.2 ± 1.8	8.1 ± 1.0
II	6	16.1 ± 2.8	13.7 ± 2.3	11.7 ± 1.5	9.6 ± 2.2	6.8 ± 1.6	7.5 ± 1.1	7.7 ± 1.1
III	7	13.1 ± 1.8	12.2 ± 2.5	11.6 ± 1.3	10.5 ± 1.6	7.9 ± 1.1	8.9 ± 1.5	9.0 ± 1.2
IV	5	13.5 ± 1.4	9.9 ± 1.2	8.6 ± 1.4	8.8 ± 1.0	6.5 ± 0.9	7.0 ± 0.7	6.3 ± 0.3
V	4	15.1 ± 5.3	13.4 ± 4.6	12.8 ± 3.1	11.7 ± 1.8	15.0 ± 0.0	16.0 ± 2.5	14.7 ± 1.8
VI	6	9.2 ± 1.7	7.9 ± 1.3	9.4 ± 0.9	6.3 ± 1.4			
Plasma ascorbic acid (mg./100 ml.)								
I	5		0.47 ± 0.06	0.46 ± 0.03	0.42 ± 0.04	0.47 ± 0.03	0.43 ± 0.09	0.36 ± 0.05
II	5		0.47 ± 0.06	0.27 ± 0.05	0.25 ± 0.01	0.29 ± 0.05	0.46 ± 0.04	0.42 ± 0.03
III	7		0.49 ± 0.02	0.26 ± 0.03	0.38 ± 0.07	0.33 ± 0.04	0.38 ± 0.06	0.41 ± 0.05
IV	5	0.44 ± 0.03	0.36 ± 0.04	0.33 ± 0.09	0.32 ± 0.02	0.30 ± 0.05	0.40 ± 0.08	0.50 ± 0.07
V	3		0.41 ± 0.05	0.30 ± 0.04	0.40 ± 0.00	0.39 ± 0.00	0.30 ± 0.00	0.44 ± 0.00
VI	6	0.53 ± 0.01	0.59 ± 0.03	0.33 ± 0.08	0.35 ± 0.04			

^a Group I. Whole milk plus alfalfa hay plus rumen inoculations.

Group II. Whole milk plus alfalfa hay.

Group III. Whole milk plus alfalfa hay plus grain plus rumen inoculations.

Group IV. Whole milk plus alfalfa hay plus grain.

Group V. Whole milk plus commercial calf starter.

Group VI. Whole milk only.

^b Standard error.

By the fifth week there was very little difference among all the groups. Group II, which received alfalfa hay alone without the rumen inoculations, declined in plasma ascorbic acid to the lowest level of any of the groups during the first 4 weeks. Groups III, IV and V, in which grain was included in the ration, declined sharply but recovered to a level intermediate between groups I and II by the twenty-first day of age.

In view of these results, it was decided to investigate the possible effects of the addition of grain to the ration of older calves which had been fed hay as the only dry feed. Four calves from groups I and II were continued on whole milk plus alfalfa hay to an average of 64 days of age.

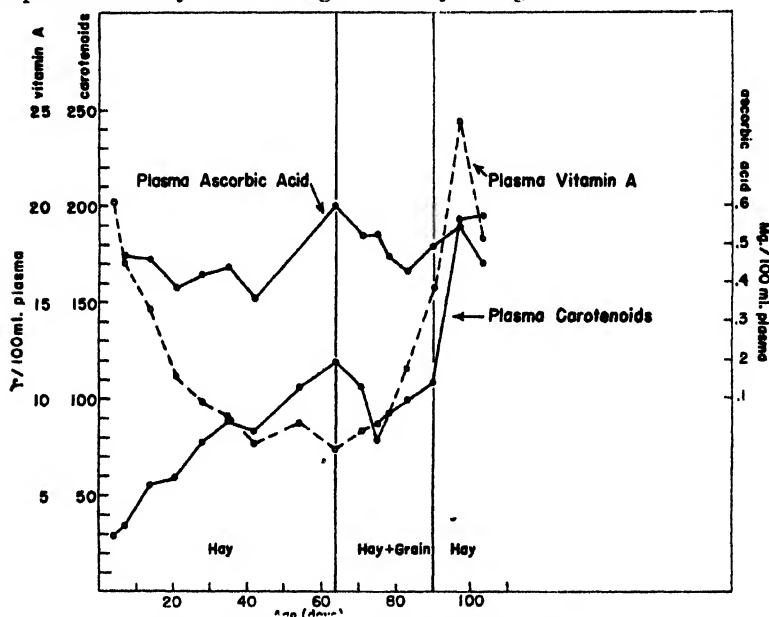


FIG. 1. The influence of adding grain to the ration of 64-day-old calves fed only whole milk and alfalfa hay until that time.

As shown in figure 1, the average plasma carotenoids had increased consistently from 29 γ per 100 ml. at 4 days of age to 118 γ per 100 ml. at 64 days of age. Plasma vitamin A had declined from 20.2 to 7.4 γ per 100 ml. during the same period, and plasma ascorbic acid values had increased from 0.47 to 0.60 mg. per 100 ml.

At this point, a 14 per cent protein grain mixture was included *ad libitum* in the ration. The week prior to the change in ration the calves were eating an average of 2.4 lb. of hay per day. Hay consumption decreased during the grain feeding period. After 4 weeks of grain feeding, the calves were eating 1.7 lb. of hay and 2.6 lb. of grain per day. Grain then was removed from the ration, and during the following week the calves consumed an average of 2.6 lb. of hay per day.

Figure 1 indicates the changes that occurred in the levels of blood plasma carotenoids, vitamin A and ascorbic acid as the result of adding grain to the ration. The plasma carotenoid level was shown to decrease, the plasma vitamin A increased and the plasma ascorbic acid decreased during this period. When grain was removed from the ration, the carotenoids rapidly increased from 108 to 193 γ per 100 ml. within a week, and the plasma vitamin A continued to rise to a peak of 24.3 γ per 100 ml. No marked changes occurred in the plasma ascorbic acid level after the grain was removed from the ration.

DISCUSSION

So far as the blood picture is concerned, the only marked beneficial effect of rumen inoculations appeared to be the higher plasma ascorbic acid level maintained in group I as compared to group II, where alfalfa hay was the only dry feed fed. The mode of action through which this effect was elicited is not readily explainable.

The suppressing action of grain feeding on the blood plasma carotenoids is strikingly demonstrated by the differences in the plasma carotenoid levels between groups I and II, which were fed hay alone, and groups III, IV and V, which received grain in addition to the hay. Accurate records of hay consumption were difficult to obtain during the first few weeks. Therefore, data are not available to demonstrate conclusively whether increased hay consumption or increased digestibility of the hay played the leading role in causing the relatively higher plasma carotenoid level of groups I and II as compared to groups III, IV and V. Indications from the data obtained were that the calves fed grain consumed less hay than those fed hay alone. It would seem that decreased digestibility of the hay possibly was a factor contributing to the low carotenoid levels observed in the grain-fed groups based on the conditions observed with respect to the microorganisms in the rumen when high proportions of grain to hay were fed (10). This would be likely especially when grain consumption reached a level equal to or higher than the hay consumption, as was the case in many instances.

It was noted that the plasma vitamin A level decreased when the plasma carotenoids were increased in groups I and II. The opposite effect on plasma vitamin A was observed when plasma carotenoids declined following the addition of grain to the ration of 64-day-old calves (fig. 1). This suggests that the plasma vitamin A level of the blood is not always a reliable indicator of the state of vitamin A metabolism in the young calf.

Sutton and Soldner (13) have presented data showing that in adult cattle the seasonal changes in blood plasma vitamin A do not closely follow blood plasma carotene changes but tend to lag behind. Plasma vitamin A often was observed to increase when plasma carotene was on the decline.

There are several factors which may be responsible for these apparent discrepancies in the behavior of plasma vitamin A in relation to the plasma carotenoids. Possibly, one of these complicating factors is the liver storage of vitamin A. The degree of saturation of the liver and whether the vitamin A

stores are being increased or depleted may influence the plasma vitamin A level, independent of the effect of the intake of carotene from the roughage. It also is possible that factors affecting the conversion of carotene to vitamin A complicate the blood picture. The answers to these questions must await further work involving the relationship between blood plasma vitamin A and liver storage, the sources of vitamin A and carotene and the physiology of the conversion of carotene to vitamin A in the calf.

SUMMARY AND CONCLUSIONS

Preliminary investigations indicated that the development of the rumen in young calves is influenced by the type of ration fed. Experiments were conducted, therefore, to determine the effect of different rations and early rumen development on the levels of vitamin A, carotenoids and ascorbic acid in the blood of young dairy calves.

Rumen inoculations, accomplished by direct transfer of cud material from cows in the herd to the calves, were supplied to about one-half the calves in order to make certain that they had access to the microorganisms present in the rumens of adult animals.

Rumen inoculations were effective in preventing the usual drop in blood plasma ascorbic acid between the seventh and fourteenth days of age when only alfalfa hay and milk were fed but were ineffective when grain was included in the ration. A ration of whole milk and alfalfa hay alone resulted in carotenoid levels considerably higher after 14 days of age than was observed when grain was included in the ration. Rumen inoculations had no marked effect on the blood carotenoid levels. Neither the inoculations nor the type of ration fed markedly influenced the blood plasma vitamin A.

When grain was introduced into the ration of 64-day-old calves, which had been fed only alfalfa hay and milk until that time, a marked reduction in hay consumption and blood carotenoids resulted. Plasma vitamin A increased and ascorbic acid declined during the same period.

These results, when correlated with the effect of different rations on the development of various rumen microorganisms in these calves, indicate that palatable, high quality hay stimulates the early development of rumen function in the young calf and appears to have a favorable physiological effect in meeting the vitamin needs of these animals.

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THE EFFECT OF SUPPLEMENTAL VITAMIN A UPON GROWTH, BLOOD PLASMA CAROTENE, VITAMIN A, INORGANIC CALCIUM, AND PHOSPHORUS OF HOLSTEIN HEIFERS^{1, 2}

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The nutritional value of vitamin A for dairy cattle has been generally accepted and numerous investigators have studied the minimum vitamin A and carotene requirements of calves and heifers for growth. Boyer *et al.* reported that 75 γ of carotene per kg. body weight was adequate for yearling Holstein heifers. Jones and Haag (5) found that heifers did not require supplemental vitamin A for growth and reproduction if they were pastured during the summer. Keyes *et al.* (6) obtained more gain in body weight by supplementing a standard calf starter with vitamin A. With this in mind, a study was undertaken to determine the value of supplementing one of the commonly used heifer rations with vitamin A. The effect of supplemental vitamin A upon blood plasma carotene, vitamin A, inorganic calcium and phosphorus concentrations and growth was studied.

EXPERIMENTAL PROCEDURE

A preliminary experiment was conducted with 22 Holstein heifers from February 1 to May 24, 1946. These animals were divided into two similar groups based upon age and body weight. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A. The vitamin A supplement used in these trials was prepared from a fish liver oil source in linseed oil meal and soybean oil meal in the amount of 250,000 USP units per lb. based on manufacturer's analysis. The basal and experimental rations were prepared with similar composition except for the supplemental vitamin A. The animals were fed mixed hay *ad libitum* and 10 lb. of grass silage and 8 lb. of a grain mixture containing 14 per cent crude protein per day. After April 1 the amount of grain was increased to 10 lb. per day.

Growth was determined by measuring body weight and height at withers of the animals. They were weighed and measured at the beginning and end of the experiment with one intermediate weight taken in April.

Blood plasma carotene and vitamin A were determined at monthly intervals using the methods of Moore (8) and Kimble (7), respectively. Blood plasma inorganic calcium and phosphorus were determined at monthly intervals using

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the Clark-Collip modifications (3) of the Kramer-Tisdall method for calcium and the method of Gomori (4) for phosphorus, respectively.

The main experiment was conducted from January 1 to May 6, 1947, using 28 Holstein heifers. These heifers were divided into two similar groups based upon age, body weight, blood plasma carotene and vitamin A, and blood plasma inorganic calcium and phosphorus concentrations. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A in the form used in the preliminary trials. The heifers were fed a weighed amount of mixed hay and grass silage at each feeding. They also received 10 lb. of grain per day.

Growth of the heifers was measured by determining the body weight and height at withers at the beginning and end of the experiment with one intermediate weight in March. Chemical analyses were the same as in the preliminary experiment, except that in the determination of blood plasma carotene and vitamin A the carotene was removed (1) previous to determining vitamin A when over 200 γ of carotene per 100 ml. were present. All data obtained in these trials were treated statistically where applicable (10).

RESULTS

In the preliminary experiment, the 11 heifers in the vitamin A group received an average daily intake of 40,382 USP units of supplemental vitamin A. This resulted in no significant differences in gain of body weight or height at withers between the two groups of 11 heifers each. The control group gained 130 lb. and 4.9 cm. and the vitamin A group gained 135 lb. and 4.9 cm.

In the main experiment the basal ration contained an average of 114,000 USP units of vitamin A per daily allowance per heifer. This was supplemented with 129,400 USP units of vitamin A per day for the 14 heifers in the vitamin A group. An analysis of variance of the gains in weight and height at withers as presented in table 1 showed a significant increase in gain in weight but no significant difference in gain in height at withers resulting from feeding supplemental vitamin A. The vitamin A group of 14 heifers gained an average of 235.9 lb. and 9.4 cm. while the control group gained 187.6 lb. and 8.4 cm., or a difference of 48.3 lb. and 1.0 cm. The heifers in the vitamin A group exhibited better condition, being smoother throughout and showing more flesh. The hair was glossier and smoother, and the hides seemed to be more pliable than those of the control group.

Feeding supplemental vitamin A at either level increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of the heifers. The mean blood plasma vitamin A concentrations of the heifers used in the preliminary trial were 18.14 γ per 100 ml. for the control group and 21 γ per 100 ml. for the vitamin A group during this trial. Similarly, during the main trial the mean blood plasma vitamin A concentrations were 15.82 and 21.71 γ per 100 ml. for the control and vitamin A-fed groups, respectively. These differences were highly significant statistically. The data from the main trial are presented in table 2. During the course of this trial, the blood plasma vitamin

A concentrations of the control group increased an average of 1 γ of vitamin A per 100 ml., whereas that of the vitamin A group increased 9 γ per 100 ml. The mean for each group was 14 γ of vitamin A per 100 ml. at the beginning of the trial.

The feeding of supplemental vitamin A resulted in a depression of the blood plasma carotene concentrations. In the preliminary trial, the blood plasma carotene concentration of the vitamin A-fed group decreased from 183

TABLE 1
Growth of heifers during main experiment

Heifer no.	Body weight			Height at withers		
	Initial	Final	Gain	Initial	Final	Gain
	(lb.)	(lb.)	(lb.)	(cm.)	(cm.)	(cm.)
Control group						
729	700	875	175	120	124	4
732	668	800	132	113	120	7
734	749	967	218	120	128	8
737	565	716	151	113	120	7
738	600	800	200	115	121	6
740	513	732	219	112	120	8
742	637	871	234	110	120	10
744	526	675	149	106	117	11
747	513	700	187	106	115	9
749	456	579	123	106	115	9
752	374	622	248	100	110	10
755	404	610	206	101	106	5
756	334	513	179	96	107	11
757	344	550	206	94	107	13
\bar{X}	527	715	188	108	116	8
Group fed vitamin A						
731	766	908	142	121	126	5
733	716	1027	311	117	123	6
735	732	1069	337	116	124	8
736	668	930	262	110	118	8
739	668	970	302	114	120	6
741	555	750	195	108	116	8
743	637	890	253	109	119	10
745	501	700	199	109	121	12
746	489	725	236	105	117	12
748	539	755	216	104	118	14
751	404	615	211	101	111	10
753	384	560	176	99	110	11
754	344	593	249	100	109	9
759	275	489	214	93	106	13
\bar{X}	548	784	236	108	117	9

to 96 γ per 100 ml., whereas the control group decreased from 220 to 153 γ per 100 ml. During the main trial, the blood plasma carotene concentrations decreased from 278 to 156 and from 271 to 85 γ per 100 ml. of blood plasma for the control and vitamin A-fed groups, respectively. Analyses of variance proved this difference to be highly significant statistically.

Blood plasma carotene and vitamin A determinations were continued during June, July and August, while the cattle were on pasture after the preliminary experiment, to determine if there was a carry-over effect from feeding supple-

TABLE 2
The effect of supplemental vitamin A upon blood carotene, vitamin A, calcium and phosphorus

Heifer no.	Carotene				Vitamin A				Calcium				Phosphorus			
	Nov. 26	March 11	May 6		Nov. 26	March 11	May 6		Nov. 26	March 11	May 6		Nov. 26	March 11	May 6	
	(γ/100 ml.)				(γ/100 ml.)				(mg./100 ml.)				(mg./100 ml.)			
					Control group											
729	393	175	153	17	19	15	8.80	8.63	8.28	8.10	8.63	7.15	8.10	8.63	7.15	
732	650	184	134	13	16	13	9.80	9.18	9.31	9.18	9.31	7.56	9.18	7.94	7.56	
734	260	142	140	21	24	21	8.93	8.18	8.68	10.11	8.68	9.18	10.11	8.69	9.18	
737	501	232	178	11	20	13	10.17	8.25	8.68	10.39	8.75	8.32	10.39	8.75	8.32	
738	375	119	108	18	16	17	9.11	9.05	9.08	9.06	9.12	8.21	9.06	9.12	8.21	
740	404	190	170	12	18	27	9.36	9.67	9.72	10.11	9.03	7.35	10.11	9.25	7.35	
742	210	246	199	13	18	13	10.11	9.05	9.72	9.44	8.81	6.52	9.44	9.30	8.57	
744	175	184	178	12	16	17	9.73	9.36	9.40	8.93	8.81	8.57	8.93	10.54	7.93	
747	307	236	167	17	15	18	9.67	8.74	8.91	8.57	10.54	7.72	8.57	10.54	7.72	
749	156	187	151	4	13	9	9.49	9.55	9.72	9.63	8.69	8.45	9.63	9.72	8.45	
752	94	131	126	16	11	13	9.35	9.11	9.66	9.97	10.25	8.93	9.97	10.25	8.93	
755	94	134	151	19	13	14	9.24	9.55	8.80	10.04	11.21	9.44	10.04	11.21	9.44	
756	148	151	164	15	14	15	9.80	9.67	9.14	9.13	9.57	8.45	9.13	9.57	8.45	
757	123	156	159	14	11	12	9.53	9.10	9.15	9.32	9.45	8.13	9.32	9.45	8.13	
\bar{X}	278	176	156	14	16	15										
					Group fed vitamin A											
731	634	96	108	17	18	19	8.93	9.05	9.14	9.70	6.76	6.72	9.70	6.76	6.72	
733	522	116	112	23	19	18	8.56	8.87	8.51	9.18	7.88	6.81	9.18	7.88	6.81	
735	398	100	105	20	22	24	9.73	9.18	9.14	7.46	8.75	6.47	7.46	8.75	6.47	
736	466	156	134	17	28	34	9.61	9.61	9.26	7.05	7.88	8.10	7.05	7.88	8.10	
739	561	114	85	20	26	40	9.42	9.55	9.08	8.81	7.67	6.95	8.81	7.67	6.95	
741	149	112	67	1	15	12	9.61	9.11	9.89	7.25	9.30	7.20	7.25	9.30	7.20	
743	183	137	105	13	26	22	9.49	9.30	9.89	8.10	6.86	6.76	8.10	6.86	6.76	
745	123	103	76	7	18	19	10.11	9.92	9.77	10.39	9.44	7.72	10.39	9.44	7.72	
746	224	98	74	11	18	20	10.11	8.87	8.97	6.81	9.44	6.66	6.81	9.44	6.66	
748	123	69	52	7	21	24	9.73	9.49	9.08	9.70	9.57	7.15	9.70	9.57	7.15	
751	96	78	63	17	17	23	9.80	8.93	10.23	9.97	9.37	7.56	9.97	9.37	7.56	
753	96	33	48	13	13	20	9.42	9.42	9.43	8.57	7.88	7.35	8.57	7.88	7.35	
754	105	80	63	16	20	23	9.80	10.04	9.31	10.39	9.18	7.15	10.39	9.18	7.15	
759	110	91	94	15	19	30	8.99	8.68	9.49	8.95	9.97	8.93	8.95	9.97	8.93	
\bar{X}	271	99	85	14	20	23	9.52	9.29	9.37	8.74	8.57	7.25	8.74	8.57	7.25	

mental vitamin A. It was found that the heifers receiving supplemental vitamin A had lower blood plasma carotene and vitamin A concentrations while on pasture than the heifers that did not receive supplemental vitamin A. The mean blood plasma vitamin A concentrations were 22.15 γ per 100 ml. for the control group and 17.45 γ per 100 ml. for the vitamin A group. This difference was significant. The mean blood plasma carotene concentrations were 874 γ per 100 ml. for the control group and 696 γ per 100 ml. for the vitamin A group. This difference was highly significant.

Feeding supplemental vitamin A had no effect upon the blood plasma inorganic calcium and phosphorus concentrations of the heifers. In the preliminary experiment there were no significant differences between the two groups; however, both groups had higher blood plasma inorganic calcium concentrations and lower blood plasma inorganic phosphorus concentrations during the summer pasture period than during the feeding trial. In the main experiment there was no significant difference in the blood plasma inorganic calcium concentrations of the two groups of heifers, but the control group had a higher (highly significant) mean blood plasma inorganic phosphorus concentration than the vitamin A group. Too much emphasis must not be placed upon this, however, since the control group had a higher mean blood plasma inorganic phosphorus concentration than the vitamin A group at the start of the trial.

CONCLUSIONS

Feeding an average of 40,400 USP units of supplemental vitamin A per day to Holstein heifers receiving a normal ration resulted in no increase in the rate of growth. Increasing the vitamin A supplement to an average of 129,400 USP units per day in addition to the 114,000 USP units supplied daily in the basal ration resulted in a significant increase in body weight gains of Holstein heifers.

Feeding supplemental vitamin A significantly increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of Holstein heifers.

Blood plasma inorganic calcium and phosphorus concentrations were not altered by feeding supplemental vitamin A.

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ASSOCIATION ANNOUNCEMENT

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Atlantic City, N. J.—October 25, 1948

Teams from twenty-six State Agricultural Colleges, participated in this, the fourteenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Donald R. Moore, Michigan State College
2. Donald H. Pfueger, Iowa State College
3. W. E. Shiffermiller, Ohio State University
4. William H. Hoagland, University of Connecticut
5. Richard E. Lewis, Ohio State University
6. John N. Lewis, Ohio State University
7. Charles H. Fitch, Iowa State College
8. Lester Hankin, University of Connecticut
8. N. V. Kennedy, Mississippi State College
10. Arnold D. Nelson, Iowa State College

Teams

1. Iowa State College
2. Ohio State University
3. Michigan State College
4. Mississippi State College
5. University of Tennessee
6. University of Connecticut
7. University of Maryland
8. University of Massachusetts
9. Pennsylvania State College
10. Cornell University

BUTTER

Individuals

- | | |
|----------------------------------------------------|-------|
| 1. Donald H. Pfueger, Iowa State College | 4.33 |
| 2. Donald R. Moore, Michigan State College | 8.17 |
| 3. William H. Hoagland, University of Connecticut | 9.00 |
| 4. William C. Flynt, Jr. Mississippi State College | 10.42 |
| 5. Delmer A. Boyce, University of Tennessee | 11.50 |
| 6. Charles H. Fitch, Iowa State College | 11.75 |
| 7. Harold McCracken, University of Tennessee | 11.84 |
| 8. William R. Vial, Purdue University | 12.25 |
| 9. Richard E. Lewis, Ohio State University | 12.67 |
| 10. John N. Lewis, Ohio State University | 13.50 |

Teams

1. Iowa State College	34.58
2. Ohio State University	39.84
3. Mississippi State College	41.92
4. Michigan State College	42.34
5. University of Tennessee	43.51
6. University of Connecticut	44.00
7. Purdue University	50.59
8. University of Nebraska	53.34
9. North Carolina State College	56.25
10. Cornell University	57.25

CHEESE

Individuals

1. William J. Deisley, Pennsylvania State College	28.17
2. John N. Lewis, Ohio State University	28.59
3. Arnold D. Nelson, Iowa State College	29.50
4. Donald R. Moore, Michigan State College	29.75
5. Ralph Whitehead, Michigan State College	31.25
6. Harold A. Newlander, Cornell University	31.34
7. Henry H. Sprowls, Texas Tech.	33.00
8. Donald H. Pflueger, Iowa State College	33.09
9. Robert J. Schuttrumpf, University of Maryland	33.43
10. M. V. Kennedy, Mississippi State College	33.50

Teams

1. Michigan State College	94.84
2. Iowa State College	98.09
3. Cornell University	100.50
4. Ohio State University	102.35
5. Texas Tech.	106.85
6. Mississippi State College	110.34
7. University of Tennessee	111.18
8. University of Illinois	112.58
9. University of Connecticut	117.50
10. Purdue University	120.52

ICE CREAM

Individuals

1. Donald H. Pflueger, Iowa State College	29.00
2. John A. McLeod, Jr. North Carolina State College	31.51
3. Ralph Whitehead, Michigan State College	32.00
4. W. E. Shiffermiller, Ohio State University	33.00
5. Donald R. Moore, Michigan State College	33.50
6. George H. Brink, University of Tennessee	34.50
6. Roland I. Zeller, University of Minnesota	34.50
8. Lester Hankin, University of Connecticut	35.00
8. William H. Hoagland, University of Connecticut	35.00
10. Delmer A. Boyce, University of Tennessee	35.50

Teams

1. University of Tennessee	108.50
2. Iowa State College	110.00

3. Ohio State University	111.50
4. University of Minnesota	114.00
5. Pennsylvania State College	116.00
6. Michigan State College	117.00
7. University of Connecticut	118.50
8. University of Nebraska	118.50
9. University of Massachusetts	127.17
10. University of Maryland	129.00

MILK

Individuals

1. William J. Deisley, Pennsylvania State College	14.92
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3. Dan Guyer, Oklahoma A. & M.	22.00
4. Luther O. Meadows, Mississippi State College	23.25
5. William H. Hoagland, University of Connecticut	23.95
5. Donald R. Moore, Michigan State College	23.95
7. W. E. Shiffermiller, Ohio State University	24.35
8. Richard E. Lewis, Ohio State University	25.20
9. Charles D. Spencer, University of Maryland	25.35
9. Alan D. Young, University of Massachusetts	25.35

Teams

1. Iowa State College	76.43
2. Ohio State University	78.45
3. Pennsylvania State College	81.42
4. University of Maryland	85.70
5. University of Massachusetts	86.37
6. Mississippi State College	86.90
7. University of Connecticut	88.65
8. Michigan State College	90.83
9. Oklahoma A. & M.	93.87
10. University of Illinois	94.92

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JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

1. **Henrici's molds, yeasts, and actinomycetes.** 2nd Ed. C. E. SKINNER, C. W. EMMONS, AND H. M. TSUCHIYA. 409 pp. \$5.00 John Wiley and Sons, Inc., New York, N. Y. 1947.

The authors have completely rewritten and considerably expanded the original edition, written in 1930. Their efforts have been directed toward the preparation of a book on yeasts, molds, and actinomycetes chiefly for students of bacteriology. In addition to expansion of previous chapters, new chapters have been included on variations in the lower fungi, infectious caused by molds, pathogenic yeast-like fungi, and antibiotic substances. The revisions in classification are in some respects quite radical. Some of the suggestions undoubtedly will meet the approval of most mycologists, but others probably will meet considerable opposition. Examples of proposed changes in generic and species designation for some common dairy microorganisms are: *Geotrichum candidum* for the mold now known as *Oospora lactis* or *Oidium lactis*, *Cryptococcus sphaerica* for the yeast known as *Torula sphaerica*, *Scopulariopsis brevicaulis* for *Penicillium brevicaulis*.

The treatment of the various individual species of molds, yeasts, and actinomycetes is necessarily brief. In view of the present lack of any adequate source of information on many such organisms and their increasing importance in recent years, it is hoped that in future editions the authors will expand their descriptions and discussions in this field.

P.R.E.

2. **Practical emulsions.** 2nd Ed. H. BENNETT. 568 pp. \$8.50. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1947.

After a general discussion of emulsions and emulsifying agents, specific technical applications are discussed by the author and by others, whose papers are presented in the form of a symposium on emulsifying agents and emulsions in industry. Lists of commercial surface-active agents, emulsifying agents, emulsions, and demulsifying and defoaming agents are given, with references to the original literature. A long section is concerned with formulas for emulsions for specific purposes. The sections concerned with food contain little of direct applicability to the dairy industry. F.E.N.

3. **The chemical composition of foods.** 2nd Ed. R. A. McCANCE AND E. M. WIDDOWSON. 156 pp. \$3.75. Chemical Publishing Co., Inc., New York, N. Y. 1947.

The data presented are the results of studies carried out by the authors

and their associates in Great Britain over a considerable period of time. The data are presented in two series of tables, one based upon 100-g. samples and one on 1-oz. samples, and recipes for the cooked foods analyzed are given in a separate section. Data on vitamin content are not given. Water, sugar, starch and dextrins, total nitrogen, protein, fat, available carbohydrate, 9 minerals, caloric value, and "acid-base balance" are given for each food. The various foods are arranged in groups, one of which consists of dairy products. F.E.N.

4. **Food products.** SAUL BIUMENTHAL. 986 pp. \$12.00. Chemical Publishing Co., Inc., New York, N. Y. 1947.

This book is a compendium of formulas and procedures for the manufacture of a great variety of food products. A chapter is devoted to milk and milk products, and these materials are mentioned in a number of other places. Although the book contains a considerable amount of information, numerous errors are apparent, some of the material definitely is out of date and the treatment is spotty. Pages are devoted to a particular process, patent or minor product, whereas a topic as important as pasteurization of milk is dismissed with only cursory remarks and practically no specific information, and the same is true of a number of other important dairy products. A 5% DDT solution is recommended for insect control, but no information concerning formulation or specific methods of application is given. The sections of microbiology and the relationship of microorganisms to sanitation are very incomplete. Many of the references given in the brief bibliography found in the appendix have been superseded by more recent material. F.E.N.

BACTERIOLOGY

5. **Observations on the use of a modified direct microscopic method for estimating bacterial quality of raw and pasteurized milk.** G. W. WATROUS, JR., AND F. J. DOAN, Pennsylvania Agr. Expt. Sta., State College. *J. Milk and Food Technol.*, 10, 5: 269-275. Sept.-Oct., 1947.

A modification of Newman Lampert formula no. 2 was recommended by using only 20% of the methylene blue dye and 20% of the glacial acetic acid as originally recommended. This stain gives a clear, light blue background, free of debris, with bacteria retaining the stain so tenaciously that identification easily was accomplished.

A ratio of one to one was attained with the modified stain and Levowitz's strip-counting technic on samples of raw milk when the plate and direct microscopic count were compared. There is no appreciable relationship

between this microscopic clump count and the plate count of organisms in fresh, laboratory-pasteurized milk. Non-viable organisms are capable of stain retention to a varying degree, depending on the microflora of the milk. Microscopic clump counts on fresh laboratory-pasteurized milk are lower than those prior to pasteurization, indicating that all the cells destroyed by pasteurization are capable of staining. A correlation exists between a high microscopic count in raw milk and the same milk after pasteurization. This relationship is not so striking where the bacterial content is relatively low in the raw and pasteurized milk. H.H.W.

6. El B.C.G. en el Uruguay. Catorce años de experiencia sobre pasajes continuados en papa-bilis-glicerizada. (The B.C.G. in Uruguay. Fourteen years of experience with continuous passage on potato-bile-glycerine medium.) A. TORTORELLA. Rev. med. vet., 4, 43: 712-717. May, 1947.

A summary of a series of experiments that have been carried out on the B.C.G. bacillus for 14 yr. is given. The B.C.G. is not a changeable bacillus and therefore may be considered a definite strain. The successive passage of the organism in bile-potato-glycerine medium influenced neither the vitality of the bacillus nor its antigenic properties. The vaccine prepared from the B.C.G. strain, which had been grown and transferred in bile-potato-glycerine medium, showed a definite activity which was reflected in the following facts: (a) It grew abundantly when cultured; (b) it produced characteristic lesions in experimental animals, but these lesions were not progressive and the animals lived; (c) it produced positive tuberculin allergy in the guinea pigs used for the experiment, even 1 yr. after the inoculation; (d) it formed specific antibodies in the blood serum of experimental rabbits. R.E.M.

7. La acción inhibitoria de la piocianina frente a las *Brucellas abortus*, *suis* y otros agentes. (The inhibitory action of pyocyanin against *Brucella abortus*, *suis* and other agents.) N. PRADINES, Brazil. Rev. med. vet., 4, 43: 718-726. May, 1947.

The organism *Pseudomonas pyocyanea* inhibited the growth of both *Brucella abortus* and *Brucella suis* in culture media. Pyocyanin, a water soluble blue pigment produced by the *Pseudomonas* organism, was found to have, by itself, the same action against the brucellas. This pigment had bacteriostatic and probably bactericidal, but not bacteriolytic, effects against those organisms. When tested *in vitro*, pyocyanin did not alter the phagocytic action of the white blood cells and retained its antibiotic action against the brucella organisms in the presence of blood serum. It also seemed to retain that property when tested *in vivo* in a chicken embryo.

Fluorescein, hemipyocyanin, and the antigen lipid-glucide, other products of *P. pyocyanea*, did not show any antibiotic action *in vitro* against the brucella species under consideration.

Preliminary experiments for a therapeutic value of pyocyanin *in vivo* against *B. abortus* and *B. suis* were carried out. The inoculation of pyocyanin did not cause any disturbances or lesions in small laboratory animals. The pigment easily was eliminated by the urine. Studies along this line still are being carried out and probably will be reported in the future. Pyocyanin also was found to have antibiotic action against *Pasteurella avicide*.
R.E.M.

8. Growth of *Staphylococcus aureus* in various pastry fillings. W. H. CATHCART, W. J. GODKIN, AND G. BARNETT. The Great A & P Tea Co., New York, N. Y. Food Research, 12, 2: 142-150. March-April, 1947.

Commercial dry-mixed puddings containing milk were found to support the growth of cultures of pathogenic *Staphylococcus aureus* when this organism was inoculated into them and the temperature made favorable. The organism was inhibited when water was substituted for the milk, but the quality of the pudding suffered. Vanilla, pumpkin, squash, and sweet potato pie fillings, cheese cake fillings, and whipping-cream mixes also were found to support the growth of this organism. By using citric acid to lower the pH of vanilla fillings to 3.43-3.65, the growth of the inoculant was checked but the filling acquired a sour taste. A better-tasting cheese cake filling was had by using lactic acid in place of citric; the staphylococcus was effectively inhibited at pH 4.42 to 4.67.
F.J.D.

BUTTER

9. Studies on the neutralization of cream for buttermaking. VII. Neutralization in practice in the butter-factory. F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 38A, 2: 132-138. Aug., 1946.

The amounts of sodium bicarbonate needed to neutralize creams of different acidities are presented in a chart and techniques employed during neutralization are described.
W.C.F.

10. Control of moisture content of butter during butter manufacture. F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 38A, 1: 31-37. June, 1946.

The formula for calculation of the amount of water to be added to

partially-worked butter in a churn to bring the water content to a definite limit is as follows:

$$\text{Quantity to be added} = \frac{\text{desired H}_2\text{O content (\%)} - \text{actual H}_2\text{O content (\%)}}{100 - \text{actual H}_2\text{O content (\%)}} \times \frac{\text{estimated load of butter in churn}}{1}$$

A chart calculated on the basis of this formula is presented; it is not applicable for calculation of salt to be added. Factors affecting the amount of water to be added are discussed. W.C.F.

11. Land-cress taint in cream and butter. Parts I and II. F. H. McDOWALL, I. D. MORTON, AND A. K. R. McDOWELL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 5: 305-315. Feb. 1947.

Land-cress (*Coronopus didymus*) is an annual weed, of the family *Cruciferae*, which appears in young pastures and in bare patches in old pastures. When consumed by cows, it imparts an off flavor to milk and cream, butter, and cheese derived from it. Both land-cress and garden-cress contain a mustard-oil glucoside and both yield benzyl cyanide on direct steam distillation and benzyl isothiocyanate by the silver salt method. The distillate of land-cress also has a peculiar burnt odor. W.C.F.

12. Land-cress taint in cream and butter. Part III. Relation of conditions of feeding land-cress to cows to incidence of land-cress taint in cream and butter. F. H. McDOWALL, I. D. MORTON, AND J. J. O'DEA, Dairy Research Inst. (N. Z.), Palmerston North, AND A. V. ALLO, Dept. of Agr., Tauranga. New Zealand J. Sci. Technol., 28A, 6: 370-384. April, 1947.

Consumption of as little as 2 oz. of cress by cows caused taint in the cream from milk drawn within 30 min. from the time of eating. When cows ate the cress at noon, the taint was strong in the cream of milk drawn 9 hr. later and was present in milk drawn the following morning. Thus, removal of cows from pastures containing the cress did not prevent the taint in the following milkings but did cause a decrease in the intensity of the defect. Land-cress hay caused a weak taint in the cream. Measures are described for the prevention of the growth of the cress in pastures. If the weed is present, only dry stock should be pastured there. W.C.F.

13. The washing of butter and its effect on curd content and quality. E. G. PONR, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 432-437. Nov., 1946.

See Abs. 279, J. Dairy Sci., 30, 9: A122.

CHEESE

14. A practical system for determining the premium value for high fat Cheddar cheese. A. B. EREKSON, Plymouth, Wis. *Natl. Butter Cheese J.*, 38, 9: 44. Sept., 1947.

This discussion is a development of a previous article. (See Abs. 311, *J. Dairy Sci.*, 29: A144. 1946.) It shows that when a working standard of 52.5% fat in the dry matter of the cheese is adopted, higher amounts of fat command a premium and lower amounts a deduction from the market price of the cheese. The value of the fat is obtained by: (a) multiplying the price per pound of 92 score butter by 1.15 to get the approximate value of fat in cream; and (b) deducting the value of 1 lb. of cheese solids. This latter value is calculated by dividing the price of cheese by 0.61, the minimum amount of dry matter permitted by law in a lb. of Cheddar cheese. A table showing the value of fat for numerous butter and cheese price combinations is given. W.V.P.

15. Factors influencing the texture of Cheddar cheese. G. H. WILSTER, Oregon State College, Corvallis. *Natl. Butter Cheese J.*, 38, 10: 48. Oct., 1947.

The texture of cheese is improved by using high-grade milk, even though the milk is pasteurized before making the cheese. Other essentials for cheese of the best texture are: clean, active starter, efficient pasteurization, clean equipment, acidity control, moisture control, and control of temperatures and pressures during cheddaring and pressing of the curd. These means for improving and controlling texture are suggested for treatments of specific defects, including gassy, greasy, open and crumbly texture, and corky and mealy-pasty body and texture. W.V.P.

16. Pasteurização a jato direto. (Direct steam pasteurization.) J. A. RIBEIRO. *Boletim do leite*, 1 (4ª Epoca), 1: 9-14. July, 1947.

A direct steam pasteurization process to be employed for milk that will be used in the manufacture of cheese is described in detail and a few drawings are given to illustrate it. The advantages credited to this process are: (a) It is a cheap form of pasteurization. (b) It is easy to use. (c) It uses a limited space in the cheese plant. (d) It takes advantage of the vacuum created by the steam injection to raise the milk to higher levels, saving time and human effort. Studies have not been made of the comparative bacteriological efficiency of the procedure. R.E.M.

17. Fermentos para queijos. O fermento láctico é a alma do queijo. O emprego de fermento perfeito é a base da obtenção de queijos.

ótimos. (Ferments for cheese. The lactic ferment is the foundation of the cheese. The use of the perfect ferment is the base in the obtention of optimum cheeses.) J. A. RIBEIRO. Boletim do leite, 1, 9: 1-4, 18. June, 1947.

A brief and general discussion of the different organisms that influence the ripening of the different types of cheeses is given. A recognized method for the day-to-day preparation of the starter, for the manufacture of semi-hard cheeses from pasteurized milk, is presented in detail. The aseptic handling of the cultures throughout the preparation of the starter is emphasized and the beneficial effects of the use of starter in the manufacture of semi-hard cheeses from pasteurized milk are enumerated. R.E.M.

18. Temperature and humidity control in cheese-curing rooms. T. R. VERNON, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 6: 361-369. April, 1947.

Cheese was of better quality after storage in an insulated room controlled automatically at 55° F. and a humidity of 85% than after storage in an uninsulated room or an insulated room without such controls. Some trouble with mold growth was encountered in the controlled room, but weight losses were less. W.C.F.

19. Control measures against the cheese-mites, *Tyrolichus casei* Ouds. and *Tyrophagus longior* Gerv. J. MUGGERIDGE, Plant Research Bureau, Nelson, AND R. M. DOLBY, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 1: 1-30. June, 1946.

Wax protected cheese somewhat against mites, but they were able to bore through the wax layer and enter the cheese. Dusts were ineffective against attacks by mites, and the high humidity necessary in cheese warehouses made the use of dusts difficult. Three fumigants, ammonia, methyl bromide, and dichloroethyl ether, were found effective, with the latter considered the most suitable. It is effective in low concentrations, is easily handled and applied, and is cheap. The chemical may be used in the vapor state as a fumigant, but the authors recommend that it be applied as a liquid to the shelves in the curing room at the rate of 1 lb. per 1,000 cu. ft. of room space or per 100 sq. ft. of shelving. Another method is to place the cheeses on boards treated with the chemical. Methyl bromide is expensive and is not as persistent as dichloroethyl ether. Ammonia is too readily absorbed by surrounding materials. W.C.F.

20. Cheese and its relation to disease. CURRENT COMMENT. J. Am. Med. Assoc., 135, 11: 718. Nov. 15, 1947.

This is a detailed review of the paper of the same title by F. W. Fabian, Am. J. Pub. Health, 37: 987. Aug., 1947.

CHEMISTRY

21. The thiamin, riboflavin and niacin content of some New Zealand milks. F. H. McDOWALL, Dairy Res. Inst. (N. Z.), N. O. Bathurst, Plant Chem. Laboratory, AND I. L. CAMPBELL, Dairy Research Inst., Palmerston North. New Zealand J. Sci. Technol., 28A, 5: 316-328. Feb., 1947.

Thiamin was estimated by the fluorimetric method and niaqin and riboflavin were measured by microbiological methods. The thiamin and riboflavin contents of Jersey milks regularly were higher than those of Friesian milks, but the niacin content was the same for both breeds. The feeding of concentrates to cows on pasture caused a rise in the thiamin content of milk from Jersey cows but not from Friesian cows. W.C.F.

22. The polarographic estimation of ascorbic acid in milk. DAWN R. PERRIN, Dairy Laboratory, Wallaceville, AND D. D. PERRIN, Animal Research Sta., Wallaceville, New Zealand Dept. of Agr. New Zealand J. Sci. Technol., 28A, 4: 266-272. Dec., 1946.

The technique was employed for the estimation of ascorbic acid in milk but it also could be used to measure dehydro-ascorbic acid. W.C.F.

23. The estimation of copper in cream. G. C. DEATH, F. RUTH LIGHT-FOOT, AND G. M. MOIR, Dairy Div. Laboratory, Dept. of Agr., Wallaceville. New Zealand J. Sci. Technol., 28A, 4: 273-284. Dec., 1946.

This is a modification of the filtration method used with butter. Improvements in the wet-ashing method also are described. W.C.F.

24. Determination of iron in foods and food products. W. D. POHLE, J. H. COOK, AND V. C. MEHLENBACHER. Research Laboratories, Swift and Co., Chicago, Ill. Food Research, 12, 3: 229-238. May-June, 1947.

A simple, accurate colorimetric method is described for the determination of iron in food products employing dry ashing and color development with 1, 10-phenanthroline. Phosphates and copper in normal quantities did not interfere. The method was applied to milk and dried milk satisfactorily.

F.J.D.

25. Note on the effect of phosphatide on the ferric thiocyanate method of estimating peroxide in fats. G. L. HILLS AND R. WILKINSON, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 430-431. Nov., 1946.

See Abs. 287, J. Dairy Sci., 30, 9: A124.

26. **The mechanism of the oxidant effects of commercial salt and water in butterfat.** G. L. HILLS AND J. CONOCHIE, Dairy Research Sec. J. Council Sci. Ind. Research (Australia), 19, 4: 414-429. Nov., 1946.

See Abs. 286, J. Dairy Sci., 30, A124.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

27. **A rapid method for the estimation of whey proteins as an indication of baking quality of non-fat dry milk solids.** H. A. HARLAND AND U. S. ASHWORTH, Agr. Expt. Sta., Pullman, Wash. Food Research, 12, 3: 247-251. May-June, 1947.

A method for estimating the baking quality of milk proteins in the form of dry milk, concentrated milk, and fluid milk is presented. The presence of a low concentration of whey proteins (indicating relatively high heat treatment) is shown to correlate with good baking quality. The whey proteins are determined, after the casein of the sample has been salted out, by acidifying the diluted filtrate and measuring the turbidity by means of a colorimeter. F.J.D.

28. **The utilization of buttermilk in New Zealand.** F. H. McDOWALL, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., 28A, 2: 97-132. Aug., 1946.

In 1940-1944, buttermilk from New Zealand creameries was used almost exclusively for animal feeding. Variations in the composition of buttermilk are discussed and other uses for the product are described. W.C.F.

29. **Directions for making kefir fermented milk.** L. A. BURKEY, Bureau of Dairy Ind., U. S. Dept. Agr. BDIM-Inf.-58. 4 pp. Nov., 1947.

Concise directions for handling kefir grains, for preparation of the milk and for handling of the product are given for both small and large scale manufacture. F.E.N.

FEEDS AND FEEDING

30. **The effect of the ingestion of high levels of riboflavin on the amount in the milk and urine.** P. B. PEARSON AND B. S. SCHWEIGERT, Agr. and Mech. College of Texas, College Station. J. Nutrition, 34, 4: 443-453. Oct. 10, 1947.

Riboflavin values of normal goat and sheep milk as measured microbiologically and fluorimetrically agreed closely. After feeding 2 g. of synthetic riboflavin, the microbiological procedure showed an increase in the

riboflavin content of the milk of about 26%. However, the fluorimetric procedure gave values for the milk of about 5 times greater than the microbiological technique. Growth responses of rats fed the milk agreed with the microbiologically determined values rather than the fluorimetric values.

The microbiological and fluorimetric values for riboflavin content of urine of rats fed large amounts (10 or 20 mg.) of riboflavin agreed closely. Both techniques gave similar values for urine from humans fed 20 mg. of riboflavin per day. Goats and sheep fed large amounts of riboflavin evidently metabolize some of the vitamin into a fluorescent compound which is biologically inactive. R.K.W.

31. **Vitamin A intake in cattle in relation to hepatic stores and blood levels.** P. H. FREY, R. JENSON, AND W. E. CONNELL. Colorado Agr. Expt. Sta., Ft. Collins. *J. Nutrition*, 34, 4: 421-430. Oct. 10, 1947.

Hereford steers of about 8 months of age were fed a carotene-free basal diet plus vitamin A supplements of 0, 25, 200, and 500 I.U. daily per lb. of body weight. Serum levels of vitamin A and carotene were determined at 0, 27, 83, 159, and 277 days and hepatic values at 0, 166, and 280 days. Animals receiving vitamin A supplements all grew at about the same rate. Dietary vitamin A did not exert a sparing action on hepatic stores of carotene. Vitamin A stores of the liver increased in practically a linear relationship with increased intake. Serum levels of vitamin A increased rapidly up to a daily intake of 100 I.U. per lb. of body weight, when the serum contained about 50 $\mu\mu$ per 100 ml. at 280 days. R.K.W.

FOOD VALUE OF DAIRY PRODUCTS

32. **Some studies on the nutritive value of butter fatty acids.** E. HEFTMANN, Univ. of Rochester. *J. Nutrition*, 34, 4: 455-467. Oct. 10, 1947.

No significant difference in gain of weights or efficiency of food utilization was found between two groups of young rats (5 males and 5 females in each group) when one group received butter fatty acids and the other group received the non-volatile fatty acids of butter for a feeding period of 5 weeks. Gains of weight and efficiency of food utilization were decreased only in the male rats fed hydrogenated fatty acids when one group received the butter fatty acids and the other group received partially hydrogenated fatty acids; intakes of vitamin A and D were low. In still another trial, the amounts and concentrations of vitamin A stored in the livers of rats fed preformed vitamin A were not significantly affected by complete hydrogenation of the butter fatty acids. R.K.W.

HERD MANAGEMENT

33. **The influence of certain milking-machine adjustments on the rate of machine-milking.** W. G. WHITTLESTON AND S. A. VERRALL, Animal Research Sta., Dept. of Agr., Ruakura. New Zealand J. Sci. Technol., 28A, 6: 406-416. April, 1947.

Variations in pulsator speeds (21, 42, and 84 pulsations per min.) and in vacuums (10, 14.75, and 19 in.) did not cause a significant difference in the milking rate.

W.C.F.

34. **Milking apparatus.** I. F. BENDER AND J. A. SCHMITT. (Assigned to Universal Milking Machine Co.) U. S. Patent 2,429,983, Nov. 4, 1947 (14 claims). Official Gaz. U. S. Pat. Office, 604, 1: 85. 1947.

A teat cup claw is described, the bottom of which easily may be opened for inspection. Suitable connections are provided for collecting the milk from the 4 teat cups and delivering it through a hose to a milk-collecting device. Nipples also are provided for the air which operates the teat cups alternately.

R.W.

35. **Comparative rates of production of different breeds of dairy cattle.** E. G. MISNER. Holstein-Friesian World, 44, 21: 2696-2697. Nov. 1, 1947.

A summary of 1,616 sires proved in D.H.I.A. work in 1945 shows that their daughters, arranged by breeds, averaged 178 lb. less milk, 2 lb. less fat, \$5 less energy value of product, and 0.05 more in fat percentage than their dams. The author interprets these figures to mean that: (a) the sires used are not so good as they should be to maintain the rate of production of our dairy herds or (b) the data are not fair to the bulls. A factor for correctly converting the total fat production to mature total fat production may be too low for converting milk production on young cows and may not give any consideration for decreasing test due to age.

A.R.P.

36. **The persistence of DDT on cattle.** R. H. HACKMAN, Div. of Ind. Chem. J. Council Sci. Ind. Research (Australia), 20, 1: 56-65. Feb., 1947.

Licking, either by the animal concerned or by another animal, is the most important factor causing the removal of DDT from cattle sprayed with that chemical. The amount of DDT ingested in this way is insufficient to produce toxic symptoms.

W.C.F.

37. **Stock watering tank.** C. FINE. U. S. Patent 2,430,165, Nov. 4, 1947 (3 claims). Official Gaz. U. S. Pat. Office, 604, 1: 131. 1947.

The chief feature of this tank is a tubular chamber spaced above the

bottom, which acts as a fire box for providing heat. The smoke is discharged through a chimney extending upward from the chamber. R.W.

38. **Electrical system for cattle stalls.** J. J. HANTZ. U. S. Patent 2,428,875, Oct. 14, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 603, 2: 264. 1947.

To improve the sanitary condition of dairy cattle stalls an electrical device is used to cause the cows consistently to deposit the droppings in the gutter back of the stall. The device consists of a horizontal bar, charged with a small electric current, adjustably suspended a few inches over the cow's back. If the cow is not standing in the proper position, she receives a small shock at the time the back is arched prior to evacuation, thereby causing the cow to step backward into position to deposit the droppings into the gutter. R.W.

MILK

39. **The significance of the coliform test in pasteurized milk.** P. D. DELAY, California Agr. Expt. Sta., Berkeley. J. Milk and Food Technol., 10, 5: 297-299. Sept.-Oct., 1947.

The coliform test is an aid in detecting faulty post-pasteurization handling. The results of this study indicate that well-equipped plants can, without exceeding the limits of practicability, handle milk which will be less than 1% positive for the coliform test. The present maximum level of less than 5 to 10% is maintained. The ultimate goal is to attain the 1% limit of coliform organisms in pasteurized milk by a gradual process, depending upon existing conditions in a given locality. H.H.W.

40. **Agregado de sustancias quimicas a la leche para su conservacion. (Addition of chemical substances to milk for its preservation.)** L. J. MURGULA. Rev. med. vet., 4, 43: 702-711. May, 1947.

Use of hydrogen peroxide, formol, sodium bicarbonate, oxygen, ozone, and trichloronitromethane or "microlysine", chemical substances that have been used to prevent the milk from curdling, was reviewed as a result of the recent use of "microlysine" as a milk preservative in France. Use of any one of the substances under consideration was discouraged and condemned as a backward step in milk sanitation. R.E.M.

PHYSIOLOGY

41. **The characteristics of the milk-ejection curve of normal dairy cows under standard milking conditions.** W. G. WHITTLESTON, Ruakura

Animal Research Sta., Dept. of Agr. New Zealand J. Sci. Technol., 28A, 3: 188-205. Oct., 1946.

To ascertain quantitatively what happens when cows are milked mechanically without hand stripping, the milk-ejection curves of a dozen cows were recorded throughout one season by means of a milk-flow recording apparatus. The average rate of milk flow tended to decline toward the end of the season, so that the time of milking did not decrease appreciably. The machine strippings did not increase significantly with declining yield, but the percentage of milk yielded as strippings increased. The starting time tended to increase and become erratic toward the end of lactation. No factor in the strict milking-machine procedure appeared to be harmful to the health of the udder.

W.C.F.

42. Contribucion al estudio del Calcio y Fosforo inorganico en la sangre de la vaca lechera. (Contribution to the study of inorganic calcium and phosphorus of the blood of the milk cow.) F. A. ROJAS. Agricultura tecnica, 7, 1: 20-25. June, 1947.

The amounts of Ca and P in the blood serum of 101 lactating cows, which represented different breeds and different levels of production, were determined. The blood samples were taken before the animals were allowed to eat in the morning. The Ca was found to be present in the range of 8 to 10.8 mg. per 100 cc. of blood serum, with 9.37 mg. as the average. P was present in the range of 2.70 to 7.50 mg. per 100 cc. of blood serum, with 4.77 mg. as the average. The Ca-P ratio fluctuated between 1.70 to 1 and 2.65 to 1, the average being 2.15 to 1. The Ca and P contents found were lower, in general, than those obtained by Haag and Jones in similar experiments.

R.E.M.

SANITATION AND CLEANSING

43. A study of the germicidal efficiency of can washing compounds. M. J. FOTER AND R. D. FINLEY, Research Laboratory, Pet Milk Co., Greenville, Ill. J. Milk and Food Technol., 10, 5: 257-262. Sept.-Oct., 1947.

The germicidal efficiency of 6 alkaline and 1 acid can-washing compounds was studied in 120 to 130 milk cans. The alkalinity levels were studied within different ranges. The germicidal efficiency was very slight or non-existent at an alkalinity range of 0.05 to 0.10% as Na_2O . When the alkalinity was increased to 0.15 to 0.20% as Na_2O , the germicidal effect was increased markedly with 2 of the cleaners.

When the acid can-washing compound was used, the acidity of the wash solution was neutralized by the calcium and magnesium salts of the hard water. This condition produced an alkaline reaction in the milk cans.

The germicidal efficiency of the acid cleaner was increased if the cleaner was maintained at a point at which the cans were acid.

The authors suggest that careful consideration should be given to the cleansing and germicidal efficiency of washing compounds in addition to rinsability, wetting, emulsification, water conditioning, etc. Germicides are not routinely used in mechanical can washers. Too much dependency should not be placed on the temperature of the rinse water, sterile rinse, temperature of the steam, and hot air for drying. H.H.W.

44. A study of the corrosion of tin plate by can washing compounds. R. D. FINLEY AND M. J. FOTER, Research Laboratory, Pet Milk Co., Greenville, Ill. *J. Milk and Food Technol.*, 10, 5: 263-268. Sept.-Oct., 1947.

The corrosive action on tin plate was studied by testing 8 commercial alkaline cleaners, 2 commercial acid cleaners, and 4 basic alkalis. All of the commercial alkaline cleaners and basic alkalis readily attacked tin, while the two acid cleaners had very little effect on the tin during the time of exposure. The acid cleaners in some cases showed spots of corrosion, with formation of pits. The corrosive action of sodium hydroxide adjusted to a wide concentration range had very little effect on the tin, except when a very low concentration was used. The oxygen content of the alkali solution probably plays a major rôle in tin corrosion.

The can washing alkalinity range generally used is 0.05 to 0.20% Na_2O , which is equivalent to 0.005 to 0.27% NaOH . This strength fell within the sodium hydroxide concentration range where maximum corrosion occurred. Results seem to indicate that a corrosive inhibitor, such as sodium sulfite, should be added to alkaline can washing compounds. An inhibitor would minimize alkaline corrosion of cans and also increase the germicidal action of alkalis in cleaning compounds. H.H.W.

45. The toxicity to houseflies of paints containing DDT. D. GILMOUR, Div. of Econ. Entomol. *J. Council Sci. Ind. Research (Australia)*, 19, 3: 225-232. Aug., 1946.

DDT was added to a glossy enamel, a flat oil paint, an emulsion-type paint, a water paint, and an ordinary oil paint. The most effective mixtures were a glossy enamel containing 20% DDT and a flat oil paint with 3-5% DDT. The effectiveness of the oil paints depended on the degree to which the DDT had crystallized in the film. W.C.F.

MISCELLANEOUS

- 46. Pasteurizing apparatus.** E. K. KINTNER. (Assigned to Arco Welding and Machine Works.) U. S. Patent 2,428,880, Oct. 14, 1947 (3 claims). Official Gaz. U. S. Pat. Office, 603, 2: 266. 1947.

A pasteurizer of the plate heat-exchanger type is described. A rubber gasket vulcanized to the perimeter of each plate prevents leakage. The equipment is characterized by a centrally located baffle which causes the liquid to flow up one side and down the other between any two plates, the direction being reversed between the next two plates for maximum efficiency of heating or cooling.

R.W.

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

47. **Fatty acids. Their chemistry and physical properties.** KLARE S. MARKLEY. 668 pp. Interscience Publishers, Inc., New York, N. Y. 1947.

This volume is divided into six sections as follows: A. The Nature and History of Fats and Waxes; B. Classification and Structure of the Fatty Acids; C. Physical Properties of the Fatty Acids; D. Chemical Reactions of the Fatty Acids; E. Synthesis of Fatty Acids; and F. Isolation and Identification of Fatty Acids. The material is covered in 23 chapters. The treatment is exhaustive and systematic. Numerous references to the original publications are given, and the author index permits ready reference to the publications of any one worker or group of workers. A 20-page subject index greatly improves the value of the book as a reference volume. Numerous figures, tables and chemical formulas are employed to advantage. In the opinion of the reviewer, this volume is a valuable addition to the reference literature.

F.E.N.

48. **Annual review of biochemistry.** Vol. 16. J. MURRAY LUCK, Editor. 740 pp. \$6.00. Annual Reviews, Inc., Stanford University P.O., Calif. 1947.

This volume continues the standard of excellency set by the preceding publications in the series. Among the 25 chapters, those of particular interest to people in the dairy industry probably are the following: Biological Oxidation and Reduction; Proteolytic Enzymes; The Chemistry of the Carbohydrates; The Chemistry and Metabolism of the Lipids; Phosphorus Compounds; Carbohydrate Metabolism; The Metabolism of Proteins and Amino Acids; Antioxidants; Choline; The Chemistry of the Proteins and Amino Acids; Mineral Metabolism; The Chemistry of the Hormones; Fat-soluble Vitamins; Water-soluble Vitamins; The Use of Pteroylglutamic Acid (Liver *L. casei* Factor, Folic Acid) in Clinical Studies; Nutrition; Carotenoid and Indolic Biochromes of Animals; Bacterial Metabolism; The Use of Isotopes in Biochemical Research: Fundamental Aspects; and The Chemistry of the Steroids. The volume serves admirably as a reference book which may be used easily because of the orderly arrangement of the material presented.

F.E.N.

CHEESE

49. **Sweet curd cottage cheese.** N. C. ANGEVINE, Angevine Dairy Laboratory, Springfield, Mo. Milk Dealer, 37, 1: 46, 132-138. Oct., 1947.

Following a brief history of cottage cheese, sweet curd cottage cheese

is defined as a cottage cheese of lower acidity which involves a definite method of manufacture with an actual control of acidity. It must be cut into cubes of equal size. It must be made by the use of a small amount of rennet or, better still, with good commercial cheese coagulator. The proper amount of coagulator is necessary to set up the curd firmly enough that it may be cut in cubes that will hold their shape at a whey acidity of 0.50 to 0.53%. Use of the short method whereby the cheesemaker controls his cheesemaking throughout is necessary. The author then discusses the advantages of the short method, the equipment needed and the short method procedure in detail. C.J.B.

CHEMISTRY

50. Determination of high molecular weight quaternary ammonium compounds as the triiodides. O. B. HAGER, E. M. YOUNG, T. L. FLANAGAN, AND H. B. WALKER. Rohm & Haas Co., Philadelphia, Pa. Ind. Eng. Chem., Anal. Ed., 19, 11: 885-888. Nov., 1947.

Two qualitative and three quantitative methods of analytical value are described which are based on the insolubility of the triiodides of many high molecular weight quaternary ammonium compounds in water. These triiodides are precipitated rapidly from aqueous solution, redissolved in dilute alcohol and determined colorimetrically or by titration with sodium thiosulfate or by potentiometric titration with a solution of iodine. One quantitative method is simple enough for the field use of health inspectors testing sanitizing rinse solutions; another is a versatile laboratory potentiometric method. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

51. Plastic cream—its production and uses. R. J. SPEIRS, Abbotts Dairies, Philadelphia, Pa. Natl. Butter Cheese J., 38, 12: 48. Dec., 1947.

Plastic cream, first developed about 1920, now is well established in commerce. It is used largely in ice cream and cream cheese but can be homogenized to make table cream. The baking industry accepts it reluctantly because of its bland flavor. It must be made from raw material of high quality and must be free of iron or copper contamination. The cream is heated to a minimum of 170° F. for 15 min. after the first separation and is held at 145° F. during the second separation. The proper container is a cylindrical cardboard type like a 5-gal. ice cream can. As soon as it is packaged, the cream is frozen in an air blast at -10 to -20° F., held at 0 to -10° F., and is shipped in refrigerated, brine-tank freight cars. A careful manufacturing process makes cream with from 79.5 to 80.5% fat.

Standard plate counts show very few bacteria in the finished product, although work should be done on psychrophilic types. Plastic cream must be carefully made and properly used to maintain the trade advantage it has earned in recent years.

W.V.P.

- 52. Cream separator.** C. M. WICKSTRUM. U. S. Patent 2,431,596, Nov. 25, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 604, 4: 704. 1947.

A conventional type glass milk bottle, containing milk which has been allowed to cream, is capped with an airtight cover, carrying 2 tubes. To the outer end of one is attached a rubber bulb for forcing air into the capped bottle just below the cap. The cream is forced out of the bottle through the second tube, the inner end of which is positioned just above the cream line and the outer end of which is bent downward to facilitate collection of the cream.

R.W.

DISEASES

- 53. Brucellosis as an occupational disease.** T. B. RICE. J. Am. Vet. Med. Assoc., 111, 849: 470-472. Dec., 1947.

A very high percentage of all veterinarians engaged in large animal practice already have, or have had, brucellosis. The disease easily is contracted by direct contact with infected animals or raw products from infected animals. The type of organism causing abortion in goats and swine gives a more severe reaction in humans than does the cattle strain. All possible precautions should be exercised to prevent the spread of the disease. Veterinarians, technicians and laboratory workers should wear rubber gloves in all contact work with unknown specimens. All foods and milk that are from possibly contaminated animals or areas should be pasteurized or heated before consumption. The germs easily are killed by heat and almost any sort of cooking is sufficient to insure complete safety to consumers. Brucellosis is extremely hard to diagnose in humans and, at the present time, treatment is not very effective. Vaccine treatments have not proved too effective as yet.

T.M.L.

- 54. Brucella agglutination tests and vaccination against cholera.** C. W. EISELE, N. B. McCULLOUGH, GRACE A. BEAL, Dept. of Medicine, School of Medicine, Univ. of Chicago; AND W. ROTTSCHAEFFER, Ann Arbor. J. Am. Med. Assoc., 135, 15: 983. Dec. 13, 1947.

Up to 80% of cholera-vaccinated humans exhibit brucella agglutinins but the brucellergen cutaneous test is negative. A common antigen exists between the two bacteria—the H antigen of *Vibrio comma*. Some 3 million veterans have been vaccinated against cholera. In this study 100

cholera-vaccinated persons were observed. Fifteen were in good health; 85 were patients in a U. S. Navy Hospital for various reasons, but 6 of these were judged to have no disease. Dates of vaccination were known in 74 cases and ranged from 6 to 28 months, with an average of 13 months. Examination by the Huddleson rapid-slide agglutination test revealed 54% positive in a dilution of 1:20 or higher, 41% positive at 1:40 or higher, and 20% positive at 1:80 or higher. This is compared to a value of 2% positive at a dilution of 1:25 or more on 11,000 Wassermann serums. Of these known to have been vaccinated 18 months before the brucella agglutination, 27% were positive at a dilution of 1:40 or higher. Emphasis is given to the effect cholera vaccination may have on the diagnosis of brucellosis in man.

D.P.G.

MILK

55. Future of H.T.S.T. pasteurization. C. A. WEBER, N. Y. State Dept. of Health, Albany. *Milk Dealer*, 37, 1: 144. Oct., 1947.

A brief history of H.T.S.T. pasteurization is given and some needed changes are discussed. The following predictions are made: (a) High-temperature short-time pasteurization will be the common method of pasteurization in all but the smaller plants until some entirely new method is developed. (b) The temperature may go higher and the time will be shortened, thereby reducing the size of the holding chamber. (c) Health officials and operators will demand a greater degree of standardization of design and uniformity of requirements but will welcome and accept changes of proved merit. (d) Thermal and safety controls will be more sensitive, responsive and dependable. Instruments will be simplified and combined to control and record automatically both temperature and time of treatment. (e) All surfaces contacted by the product will be streamlined and finished so that less milk solids will accumulate. Mechanical washing will be improved, thereby reducing manual labor and cleaning cost. A practical means or method of starting and finishing an operation without the intermixing of milk and water would be very desirable.

C.J.B.

56. Frozen whole milk. W. A. KRIENKE, Univ. of Ill., Urbana. *Milk Dealer*, 36, 12: 45, 68-72. Sept., 1947.

The information available at the present time would seem to indicate that, from the standpoint of processing, storage and reconstitution for fluid milk uses, frozen whole milk either in the unconcentrated or in the concentrated form has a promising future. A high quality milk supply is the first essential, as it is in market milk. A high preheating temperature of the milk (170-180° F.) is desirable. Copper contamination should be avoided. If the milk is condensed, a concentration ratio of 3 to 1 is satis-

factory, and the condensed milk should be homogenized at a pressure of 2,000 to 3,000 lb./in.² when using the piston type homogenizer. Addition of a small percentage of dextrose has no appreciable effect on the quality of frozen condensed whole milk; its use is not recommended.

The homogenized whole milk or condensed whole milk should be cooled to approximately 40° F. after processing and immediately filled into the proper containers. Effective sealing of the container is essential in order that the entire package has a relatively low moisture vapor transfer rate. Apparently freezing of the products can be done most effectively in a freezing tunnel operated with a blast of air at temperatures of -10 to -15° F. or lower. The frozen products should be transferred from the freezing tunnel directly into the storage chamber. A low storage temperature (-15° F. or lower) is very essential, and a minimum fluctuation of the storage temperature also is of great importance. If transfer of the products to a refrigerated storage having a temperature above -10° F. is necessary, complete defrosting should precede the elevated storage, which should be slightly above freezing.

Defrosting and reconstitution of the frozen condensed milk should be done by placing the frozen block into the proper volume of water at 165-180° F. and allowing it to melt without agitation. For a product of the coffee cream or cereal cream type, proportionately less water should be used than for normal fluid milk. By properly adjusting the concentration ratio of the condensed milk before packaging, it will be possible to use convenient quantities of water for reconstituting a unit of the frozen condensed milk into either of these products. Specific instructions for defrosting and reconstituting should appear on the package. C.J.B.

57. Control of vitamin D milk. CURRENT COMMENT. J. Am. Med. Assoc., 134, 17: 1486. Aug. 23, 1947.

In 1933 the Council on Foods and Nutrition of the American Medical Association began to grant acceptance to bottled fresh milk fortified with vitamin D. To secure the Seal of Acceptance of the Council, dairies are required: (a) To declare on label or bottle cap the source and unitage of vitamin D, (b) to have their advertising copy approved by the Council, and (c) to submit proof to the Council in the form of bioassay reports attesting that the milk has been assayed by a reputable laboratory and found to be up to the required potency of 400 units of vitamin D per quart. Many concerns that sell vitamin D milk use the Council's Seal and abide by the regulations, but purveyors of vitamin D concentrates sometimes sell their products without mentioning the need for routine control to assure the physician and the public that proper potency is maintained. Apparently no checkups are made unless required by official agencies or by the Council. The only states having such regulations and providing bio-

logical testing of such milks are Conn., N. Y., Ky., Va. and Wis. Chicago, Cleveland, Detroit, St. Louis and New York are among cities requiring frequent tests. Since vitamin D milks are now depended on by physicians to supply vitamin D, it is suggested that physicians should inquire of their local or state health departments to determine whether such milks are subjected to routine control and whether such regulations exist for the protection of the public and the medical profession. D.P.G.

58. **Should we have a single standard of milk quality?** G. M. TROUT, Michigan State College, East Lansing. *Milk Dealer*, 36, 12: 134-138. Sept., 1947.

The author concludes that a single minimum standard of quality for milk would be a blessing to the dairy industry and to mankind in general. All the milk solids then could be used for human consumption rather than converting some of them to cheaper animal feeds. Many states now have such a minimum quality standard for milk, but the regulations cannot be enforced at the origin of production due to the inadequacy of personnel. The burden of enforcement cannot be borne by regulatory officials alone. It must necessarily rest in large part with the milk buyer. The legal maxim, "Let the buyer beware", soon may apply to milk buyers as it does to persons in other industries. The time is at hand when all milk solids, either for bottle or for manufacturing purposes, should meet certain minimum quality standards. C.J.B.

59. **The demand side of the milk market.** L. SPENCER, Cornell Univ. *Milk Dealer*, 37, 1: 116-122. Oct., 1947.

A discussion of the effect of exports, military use, and civilian consumption on the national consumption of dairy products during the past 6 or 7 years is presented. A report then is given on the fluid milk sales in Buffalo, Rochester, and New York City, and of the per capita consumption of milk and cream in the New York-New Jersey Metropolitan area. During the war period the U. S. Government was a heavy buyer of dairy products, taking nearly one-sixth of the total output in some years. Government buying has been reduced drastically and will not be resumed on a large scale except to prevent prices of dairy products from falling to disastrously low levels. Civilian consumption per capita of most dairy products now is much above the prewar level, due largely to high consumer incomes which stimulate the demand for choice foods. Higher retail prices since the end of price ceilings and subsidies last year, combined with increasing supplies of other products, have caused some decline in per capita purchases of fluid milk and cream and ice cream. Butter consumption has increased with the appearance of more adequate stocks but remains far below the prewar level. Per capita consumption of all butterfat is below prewar, while consumption of nonfat solids is nearly one-fourth larger.

Fluid milk sales in the cities of New York State increased about 25 to 50% between 1939 and 1946 but have suffered a slight decline during the last year. Per capita consumption of fluid milk in the New York-New Jersey Metropolitan area reached the highest level in 1945 at 0.93 pint daily. It declined to 0.90 pint in 1946 and will be lower in 1947, although still fully 10% above the prewar rate. Per capita consumption of cream in New York is at least 25% lower than the prewar rate. Even at the higher retail prices now in effect, fluid milk is cheap in relation to the enlarged incomes of industrial workers. A week's earnings of factory workers in New York City now will pay for about one-fifth more milk than could be purchased with prewar earnings. Whether the demand for milk and other dairy products will remain at or near the recent high level depends largely upon the continuance of industrial prosperity and full employment. If business activity and employment should decline, the demand for milk and other choice foods might be affected more seriously than was the case when consumption rates for these products were lower. C.J.B.

60. Campaign of Pennsylvania milk distributor explains where the milk dollar goes. D. S. ADAMS, St. Lawrence Dairy Co., Reading, Pa. Milk Dealer, 37, 1: 42, 43. Oct., 1947.

The method used by the St. Lawrence Dairy Co. to inform their customers, dairy employees, and milk salesmen of the economic factors which operate in the production, processing and delivery of milk is explained. The method is known as the eight-point consumer relations program and is as follows: Ad no. 1 as well as bottle hanger no. 1 points out that the farmer gets 60 cents out of every dollar spent by the consumer for milk. The second ad stresses the fact that dairy employees get 24.5 cents out of each dollar the consumer spends for milk. The third ad reveals that it costs 5 cents to run the dairy, 2 cents for office operation and 3 cents for plant maintenance. The fourth ad explains it cost 3.5 cents for delivery of milk, the fifth that bottles and containers cost 2 cents, the sixth that 2.5 cents is spent for employee pension and insurance funds, and the seventh that 1.5 cents is paid in taxes. The eighth and final ad of the series reveals the usually surprising information that it costs almost 99 cents to deliver a dollar's worth of milk to the consumer's door, and only 1 cent, or one-fifth cent per quart, remains for profit! Each of the ads also emphasizes the value and relatively low cost of milk. "Milk", each ad repeats, "gives you the biggest food value for your dollar." C.J.B.

61. Liquid cooling unit. D. L. KAUFMAN. (Assigned to General Motors Corp.) U. S. Patent 2,431,484, Nov. 25, 1947 (7 claims). Official Gaz. U. S. Pat. Office, 604, 4: 675. 1947.

Liquids in bulk containers, *e.g.*, milk or cream in 10-gallon cans, are

cooled rapidly, conveniently and efficiently by a device consisting of a submerged cylindrical evaporator in which a compressed refrigerant is allowed to expand and evaporate. Provision is made to mechanically raise and lower the evaporator and to agitate the liquid to be cooled by means of a propeller just below the evaporator. When not in use the evaporator is protected by a shield comprised of telescoping sections which automatically extend and contract as the evaporator is lowered and raised.

R.W.

PHYSIOLOGY

62. **The blood groups of cattle.** L. C. FERGUSON. J. Am. Vet. Med. Assoc., 111, 849: 466-469. Dec., 1947.

Repeated therapeutic transfusions in cattle often are dangerous; however, most animals can sustain at least one transfusion without clinical response. Reactions usually are characterized by muscular trembling, salivation, lacrimation, coughing, hemoglobinuria, general depression, and temperature elevation to 104-105° F. The abortion of the foetus also resulted in 3 cases. The symptoms, in general, resemble rather closely those in human cases where repeated transfusions of compatible blood are used. The severity of reaction may be decreased in some cattle by the intravenous injection of adrenalin. Genetic studies of cellular antigens have revealed several useful methods of parental identity in cattle. The most positive conclusion at the present time is that a calf may possess a particular antigen in its blood only if one or the other or both of the parents possess that antigen. This method of parentage determination already has been used to good advantage in many cases. The possibility also exists that the genes determining blood groups in cattle are linked closely with those for milk production. Investigations are under way to reveal these associations.

T.M.L.

SANITATION AND CLEANING

63. **How to clean heavily contaminated bottles.** C. M. MOORE, Cowles Detergent Co., Cleveland, Ohio. Milk Dealer, 36, 12: 43, 44, 124-132. Sept., 1947.

There are two distinct phases to bottle washing, the bottle washer representing the mechanical phase and the cleaner the chemical phase. The duty of the machine is to take the bottles to the cleaning solution, to necessary rinse or pressure jets or brushes, and then deliver the bottles to the inspection conveyor. It is the duty of the cleaner to remove the visible dirt from the bottle, to destroy bacteria, mold and yeast, and then remove itself from the bottle as quickly and completely as possible. When operated properly, the mechanical phase of bottle washing is a fairly well fixed

and established function. Minor mechanical changes sometimes can be affected, such as increasing the time of contact by slower operation, etc. The important thing is to make sure that the bottle washer is operating properly from a mechanical viewpoint, and then make such changes or adjustments as are necessary in the chemical phase. The latter changes are discussed under extraneous matter, time of contact, temperature and strength of solution. Special emphasis is placed on the use of wetting agents. Directions are given for determining the concentration or the amount of these agents to be used and for determining the quantity required for upkeep.

C.J.B.

MISCELLANEOUS

64. **Waste disposal for country plants.** T. F. WISNIEWSKI, Wisconsin State Board of Health. *Milk Dealer*, 36, 12: 50-51. Sept., 1947.

The author states that: (a) Spending money on avoiding waste and on utilizing waste products is more economical than spending it on enlarged waste disposal plants. (b) The most common method of milk waste treatment now in use, providing 80-90% reduction in biochemical oxygen demand, consists of the intermittent application of the waste to a filter composed of crushed stone. The treatment plant consists of a holding tank, trickling filter, and settling tank. The construction and operation of such a plant are described.

C.J.B.

65. **Selection and applications of trucks.** J. N. BAUMAN, White Motor Co. *Milk Dealer*, 36, 12: 41, 42, 100-112. Sept., 1947.

A tremendous waste in motor transport occurs because, in a large proportion of motor truck installations, consideration is not given to the work that is to be done so that the motor truck can be fitted accurately to the job. A truck is entitled to the same consideration in its applicability for the job as a bottling machine in its applicability. The following 8 steps or procedures, arranged in the order in which they should be considered and which, when complete, will answer all questions of truck application, are set forth and discussed: (a) Determination of the work that the truck is going to be required to do. (b) Determination of the horsepower required to perform this work properly. (c) Selection of the correct model and determination of its wheelbase and load distribution. (d) Selection of the proper tire sizes and types for most efficient operation. (e) Selection of the type of axle best suited for the work that is to be done. (f) Determination of the proper rear axle ratio to bring about the greatest over-all economy of operation. (g) Selection of the type of transmission that will give the most efficient operating condition. (h) Selection of the various optional equipment items that will make possible maximum results.

C.J.B.

JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

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ABSTRACTS OF LITERATURE

BOOK REVIEW

- 66. Food regulation and compliance.** Vol. II. A. D. HERRICK. 655 pp. \$10.00. Revere Publishing Co., New York 4, N. Y. 1947.

This is the second volume of an interpretation of the Federal Food, Drug, and Cosmetic Act as it applies to food. Special attention is given to the adulteration of foods, including that occurring during processing, packaging and shipping. Considerable space is given to problems of administration of the Act and to a description of the powers of those concerned with its enforcement. Activities connected with the control of importation and exportation of foods are discussed, as are methods of inspection of premises and sampling of products. The book describes the regulation of foods under the Act. It is quite general; yet the many examples given illustrate interpretations of specific conditions.

There is no section devoted, as such, to dairy products. They are mentioned incidentally with other types of foods. Its value to persons interested mainly in dairy products lies in the information given concerning the interpretation of the law and in the description of the methods of enforcement.

The chapter headings illustrate the subject matter discussed. They are as follows: Adulteration in Food Products, Harmful Substances in Foods, Contaminated Foods, Insanitary Premises and Processing, Deleterious Containers, Economic Adulteration, Adulteration in Confectionery, Administrative Regulations, Imports and Exports, Emergency Permit Control, Coal-tar Colors, Inspections and Sampling, Enforcement Means and Methods, Offenses and Violations, Criminal Prosecution, Seizure Proceedings, Injunctive Proceedings. There is an appendix entitled "Federal Food, Drug, and Cosmetic Act and General Regulations for Its Enforcement" in which the drug and cosmetic sections and general regulations are omitted.

M.P.B.

BACTERIOLOGY

- 67. The effect of variations in technique on the plate count of milk powders.** A. H. WHITE, Div. Bacteriology and Dairy Research, Dept. of Agr., Ottawa, Canada. *Sci. Agr.*, 27, 9: 405-413. Sept., 1947.

A study on the influence of type of diluent, temperature of diluent and temperature of incubation in the bacteriological analysis of milk powder by the plate method is reported. Spray process whole milk powders and spray and roller skim milk powders were analyzed. The use of 0.1 N

lithium hydroxide as a diluent greatly reduced plate counts, probably due to the high alkalinity or pH of the solution. Even when diluted 1:100, the pH values of reconstituted milks averaged 9.65. Reconstituting the milk with the diluent at 50° C. resulted in increased plate counts as compared to using a diluent at room temperature. An incubation temperature of 32° C. as compared to 37° C. had little effect on the counts. However, the lower temperature is recommended if available. O.R.I.

68. Sur une méthode simple d'isolement et d'étude des ferments de l'arome des beurres. (On a simple method for isolation and study of aroma-producing ferments of butter.) JEAN KEILLING AND A. CAMUS. Lait, 27, 265-266: 235-237. May-June, 1947.

Based on the premise that aroma-producing organisms develop more satisfactorily in a medium of diluted buttermilk, a method has been devised for the isolation and examination of organisms of this type. One milliliter of serum from butter to be tested is transferred to 1% sterile glucose solution at 20° C. After being shaken at intervals for 2 hr., 1-ml. transfers are made to sterile milk diluted 50, 75, 90, 95 and 98% with sterile water. These tubes are maintained at 18° C. for 24 hr. The contents of each tube then are examined microscopically and diacetyl content determined. Cultures showing desirable properties on these tests then are purified by serial dilution and propagation on solid media. O.R.I.

69. Method of carrying out fermentation processes for production of riboflavin. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,433,063, Dec. 23, 1947 (4 claims). Official Gaz. U. S. Pat. Office, 605, 4: 622. 1947.

The synthesis of riboflavin by the fermentation of sterilized whey or skim milk by *Clostridium acetobutylicum* is improved by having the iron content in excess of 0.1 and 0.21 p.p.m. and the pH maintained in a definite range depending on the quantity of iron. R.W.

70. Method of preparing riboflavin from whey and skimmilk. N. E. RODGERS, H. L. POLLARD, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,433,064, Dec. 23, 1947 (4 claims). Official Gaz. U. S. Pat. Office, 605, 4: 623. 1947.

Essentially the same as U. S. Patent 2,433,063 (see preceding abstract) except that the pH of the medium is adjusted to 5.8 to 6.5 with lactic acid prior to sterilization. R.W.

71. **Method of preparing riboflavin from lacteal material.** R. E. MEADE, N. E. RODGERS, AND H. L. POILLARD. (Assigned to Western Condensing Co.) U. S. Patent 2,433,232, Dec. 23, 1947 (6 claims). Official Gaz. U. S. Pat. Office, 605, 4: 667. 1947.

The production of riboflavin from whey or skim milk by *Clostridium acetobutylicum* is increased by incorporating xylose in the medium.

R.W.

BREEDING

72. **The causes and diagnosis of infertility in bulls.** G. R. MOORE. J. Am. Vet. Med. Assoc., 112, 850: 25-29. Jan., 1948.

The causes of sterility in the bull may fall logically into 3 groups: (a) those caused by inflammatory processes of the testes and of the tubular genitalia, (b) testicular hypoplasia and degeneration due to hormone deficiencies and other causes, and (c) functional disturbances due to systemic ill health, senility, malnutrition, obesity and lack of exercise. Certain precautions in management and breeding may greatly improve the fertility of sires. Selecting for reproductive efficiency and good fertility is extremely important in any breeding program. It also is a good management policy to provide the young sire with adequate green feeds, protein supplements, minerals, and an opportunity for an abundance of exercise for best breeding efficiency.

T.M.L.

73. **Cost of getting cow with calf.** W. F. SCHAEFER. Guernsey Breeders J., 73, 1: 11-13. Jan. 1, 1948.

The cost of keeping bulls at the Nepa Artificial Breeding Cooperative, Tunkhannock, Pa., averaged \$614 in 1946. This amount included: Lease fee and depreciation of bulls owned (depreciation 50% per year) \$222, feed \$175, labor \$150, housing \$37, bedding \$12, trucking \$11, veterinary \$7. During the year, 23,141 cows were bred at a cost of \$3.95 per cow offered for service. An average of 1.53 inseminations was required per cow.

A.R.P.

BUTTER

74. **A fluorescence method for assessing the keeping quality of butter.** G. A. GRANT AND W. HAROLD WHITE, Natl. Research Laboratories, Ottawa, Canada. Can. J. Research, F, 24: 461-466. Nov., 1946.

A method was developed for determining the fluorescence of the diluted sera of butter samples. Fluorescence values were found to be related to flavor scores of salted butters which had been stored at high temperatures. The recommended procedure was as follows: The serum was separated by placing 125 g. of butter in centrifuge bottles and heating in a boiling water bath, centrifuging at 1700 r.p.m. and siphoning off the fat. Two ml. of the

serum was diluted to 50 ml. with 10% sodium acetate, the pH adjusted to 5-6, and the fluorescence values determined immediately in a Coleman photofluorometer using a filter that transmitted light in the region of 365 m μ . This procedure gave fluorescence values that were correlated with flavor scores ($r = -0.84$) on salted butters stored at 32.2° C. (90° F.) and sampled at intervals during 32 days. O.R.I.

75. **Contribution a la connaissance du beurre de brebis (beurre de cashcaval) prepare en Roumanie. (Contribution to the knowledge of sheeps' butter (cashcaval butter) prepared in Roumania.)** C. STOIAN. *Lait*, 27, 267: 342-352. July-Aug., 1947.

In the making of cashcaval cheese from sheeps' milk, the curds are heated by hot water and high losses of fat occur. This may be salvaged either by gravity or centrifugal separation. Butter oil prepared from this fat is marketed in southeastern Roumania. Eight commercial samples were analyzed for such physical and chemical constants as melting and solidification point, index of refraction, specific gravity, saponification, Reichert-Meissl, Polenske and other values. Widely varying values were obtained in melting and solidification points, but in other respects fairly uniform values were found. The average R-M value was 29.88 and the average P value 4.53. O.R.I.

CHEESE

76. **Factors influencing the quality of cheese.** H. L. WILSON. *Can. Dairy Ice Cream J.*, 26, 11: 45. Nov., 1947.

Sanitation is the most important factor confronting the cheese industry. The operator of a clean, sanitary plant should not accept milk of inferior quality. All bacteria foreign to cheesemaking should be eliminated. The control and proper rate of acid development are important in the making of a good uniform quality of cheese. The time element also is very important; 2.25 hr. should elapse from setting to drawing the whey, and at least 2.25 hr. from time of drawing whey to milling. To make a cheese that is uniform in quality as well as type, a uniform method and time schedule must be used, varying only those steps that are necessary because of bacterial action. H.P.

77. **Rheological experiments carried out on Gruyere.** G. MOCQUOT, G. W. S. BLAIR, AND M. BARON. *Natl. Inst. for Research in Dairying, Shinfield, England. Dairy Inds.*, 12, 10: 966-976. Oct., 1947.

The measurement of superficial density used for Cheddar cheese manufacture in England has been applied to the production of Gruyere type cheese made in French Jura. The method (described in a previous publication in 1940), which measures the superficial density of the curd, was found

to be very valuable to cheesemakers, particularly when they are confronted with the processing of abnormal milks (mastitis, high acid, etc.).

Measurements for plasticity and elasticity by means of a ball compressor during curing constitute a valuable tool in predicting the future quality of cheese. With this information the cheesemaker can then correct or prevent certain defects from developing by such procedures as accelerating or retarding ripening.

D.V.J.

- 78. Apparatus for use in the centrifugal separation of serum from cheese constituents.** G. J. STREZYNSKI. (Assigned to De Laval Separator Co.). U. S. Patent 2,432,829, Dec. 16, 1947 (13 claims). Official Gaz. U. S. Pat. Office, 605, 3: 507. 1947.

A centrifuge has been developed which continuously removes whey from a cultured milk-cream mixture to form cream cheese. The mixture passes through a revolving bowl which is provided with 2 outlets, one for the lower density constituent whey and the other for the concentrated curd and fat. The movement of the latter is facilitated by a scaper or conveyor and by maintaining the incoming temperature of about 160° F. through suitable insulation of the equipment. The mechanism is so designed that the curd and fat mixture is not aerated as it continuously leaves the bowl and moves through the supplementary devices. Measured amounts of such desirable additives as gum, salt and flavoring ingredients may be continuously injected into and intimately mixed with the cream cheese as it is discharged from the machine.

R.W.

CHEMISTRY

- 79. Différenciation de la caséine et de la lactalbumine par un processus microbien.** (Differentiation of casein and lactalbumin by a microbial process.) JEAN KEILLING AND A. BARRET. *Lait*, 27, 267: 337-342. July-Aug., 1947.

In the course of studying dairy fermentations, a mycoderma was isolated which possessed the ability to digest casein to amino acids. No amino nitrogen, as determined by the Sorensen method, was produced in media in which albumin was the source of nitrogen. The organism on solid media produced small round colonies with irregular contours. The cells were elongated, 3-5 μ in diameter and 5-6 μ in length. They reproduced by budding. When cultured in milk, a maximum yield of amino nitrogen was obtained in 16 days at 30° C. The total lactose of milk was not greatly reduced after 16 days. It is suggested that this organism will be of value in laboratory determinations and in the ripening of soft cheese. O.R.I.

- 80. Analyse des crèmes.** (Analysis of creams.) R. MOREAU. *Lait*, 27, 265-266: 257-258. May-June, 1947.

A modification of the Koehler-Bacot method is proposed whereby the

sulfuric acid and water would not be added separately to the butyrometer but would be mixed in the proportion of 5 ml. of water to 10 ml. acid prior to being added. After adding amyl alcohol, the tubes are placed in a water bath at 85° C. to facilitate digestion. The fat columns are measured at 65° C.

O.R.I.

DISEASES

81. **Mastitis.** R. F. WAECHTER. *Can. Dairy Ice Cream J.*, 26, 11: 90. Nov., 1947.

Chronic mastitis, the type most frequently found, is defined as a progressive inflammation of the udder or mammary gland. From this infection, the milk secreting tissues in the gland gradually are destroyed. Mastitis can be detected by the strip cup, manipulation of the udder, or by bacteriological tests. Methods of control require hygienic stable and milking conditions. Newer methods of treatment include the use of the newer sulfa drugs and penicillin. The use of mastitis mixed bacterins in the treatment and prevention of mastitis is recommended. The advice of a veterinarian should be obtained when the disease is observed.

H.P.

82. **Streptomycin in the treatment of calf pneumonia.** R. F. VIGUE. *J. Am. Vet. Med. Assoc.*, 111, 848: 389-390. Nov., 1947.

In 7 cases of calf pneumonia, 3 of which were complicated with diarrhea, all terminated favorably after streptomycin was used in addition to blood transfusion and oral sulfadiazine therapy.

T.M.L.

83. **Treatment of pneumonia in cattle.** S. J. ROBERTS AND G. K. KIESEL. *J. Am. Vet. Med. Assoc.*, 112, 850: 34-39. Jan., 1948.

Treatment with sulfamerazine, sulfamethazine and penicillin resulted in the recovery of 94.6% of 129 cases of the pneumonia form of hemorrhagic septicemia in older cattle; 93 calves treated in the same fashion gave 81.7% recovery. Although no animals were kept untreated for checks, evidence indicates that treatment with the newer pyrimidines has been somewhat effective in reducing losses of cattle from pneumonia. When sulfamerazine and sulfamethazine were used in the recommended dosages of 0.5 to 0.75 gr. per lb. of body weight daily, no toxic reactions were observed.

T.M.L.

84. **A further report on staphylococcic abortion in a dairy herd.** W. D. POUNDEN, L. C. FERGUSON, C. E. KNOOP, AND W. E. KRAUSS. *J. Am. Vet. Med. Assoc.*, 111, 848: 376-378. Nov., 1947.

In a herd of 50 cows bred artificially to 7 different bulls, 6 out of 15 cows bred to one particular sire aborted. The time of abortion varied from 137 to 242 days. All animals that aborted were inseminated anterior to the cervix. (This procedure was used as a precaution against further in-

fection.) An organism resembling *Staphylococcus albus* was recovered from the necrosed cotyledons or pus in 4 instances. In 2 cases the organism was found in the aborted calves. Organisms of apparently similar characteristics were recovered from semen samples from the bull in question. All cows bred were negative to tests for brucellosis and trichomoniasis.

T.M.L.

ICE CREAM

85. **Overrun control in ice cream.** P. H. TRACY, Dept. of Dairy Husb., Univ. of Ill. Ice Cream Field, 50, 4: 88, 89. Oct., 1947.

The importance of overrun control is stressed and data given to show the relation of overrun to ice cream ingredient cost. However, ingredient costs are not the only ones affected by producing ice cream with lower overrun. The author states: "The aim of the industry at the present time is an ice cream with an overrun of about 60 percent." Dipped ice cream should have slightly more than 50% overrun; fewer problems result in producing packaged ice cream at an overrun of 60-65% than at 50%. Dipping materially changes the texture of ice cream. Low overrun (40-60%), machine filled packages have a better texture than hand dipped packages with the same overrun. The author suggests that ice cream be frozen to the usual 100% overrun and, after partially freezing, part of the air be pressed out so as to obtain the desired overrun. The economics of the problem requires that the industry ascertain the optimum overrun consistent with quality and the willingness or ability of the consumer to pay.

W.C.C.

86. **The shrinkage problem.** C. D. DAHLE AND J. A. MEISER, JR., Dept. of Dairy Husb., Pennsylvania State College. Ice Cream Rev., 31, 5: 64, 67. Dec., 1947.

In order to obtain information as to the prevalence of shrinkage in ice cream and to learn of methods used by the industry to combat it, a questionnaire was submitted to members of the I.A.I.C.M. The answers indicated that more shrinkage occurred in plants using sweetened condensed milk than when other forms of concentrated milks were used. The elimination of wheat sirups from the mix eliminated shrinkage in 11 out of 18 plants using this product. Eliminating or reducing the amount of other sirups also eliminated a considerable amount of shrinkage. Lowering hardening room temperatures from +5 or -10° F. to -15 or -20° F. was effective in reducing shrinkage in several cases. Freezing ice cream to a lower temperature at the freezer and slowing down the continuous freezer were found helpful in controlling shrinkage. Some plants reported they stopped shrinkage by changing stabilizers, and 3 plants stopped their trouble by eliminating the use of emulsifiers. There is no single remedy for the shrinkage problem.

From results of research work, as well as from information obtained from the questionnaire, it would appear that the use of sweetened condensed milk, the use of certain sirups in the mix, the presence of free fatty acids, the use of emulsifying agents, improper surface of paper containers, improper temperatures in the hardening room and cabinets, and season of year may contribute to shrinkage.

W.J.C.

87. **Vanilla, the edible orchid.** N. C. LARSEN, Polak & Schwarz, Inv., Ice Cream Trade J., 43, 8: 72, 73, 85-88. Aug., 1947.

This article deals with the early history relating to the discovery of the vanilla bean, definitions of the various types of beans, how they are propagated, cured and packaged, quantity consumed, composition and uses.

W.H.M.

88. **Defrosting.** S. RUPPRIGHT. Ice Cream Trade J., 43, 8: 78, 98. Aug., 1947.

Defrosting of refrigeration coils may be accomplished with outside air or with hot gas from the high side of the refrigeration system or brine in a brine system through valved connections. Another method is by use of heat of dissolution liberated by thinning a brine with the substance of the frost. Electrical resistance heat may be used when the resistance wire is thermally united with the coil. Water also is used as a carrier of heat for defrosting evaporator coils. Water defrosting may result in freeze-ups unless done quickly with special equipment. The washing effect of water is desirable and aids in removal of odors from the coils.

W.H.M.

89. **Improving packages.** J. H. ERB, The Borden Co. Ice Cream Trade J., 43, 10: 104, 144, 145. Oct., 1947.

The most common faults of much packaged ice cream are high overrun, weak body, course texture, fluffy body and poor flavor. Packaged ice cream should have a fine flavor, smooth texture and firm body. A total solids content of 38.5 to 40% is desirable. The use of corn sirup to gain in non-sweet solids and of high grade egg yolk to produce small air cells is advantageous. Only high quality raw material should be used. Thorough homogenization and 5 hr. of aging are recommended. In freezing, the overrun should be kept under that of corresponding flavors of bulk ice cream. Every container should be of the same weight. The ice cream should be frozen to a stiff dry consistency and transferred to the hardening room quickly. The hardening time should be 6 to 8 hr. Appealing flavor and flavor combinations assist in promoting the sale of packaged ice cream. In addition to standard flavors, 2-layer, 3-layer and revel type of packages are desirable. The type of container from which ice cream must be dipped yields a product more like hand-dipped bulk ice cream, but this style of package lacks the ready serving convenience of the container which is

easily pulled away from the ice cream. Another important factor in the production of satisfactory packaged ice cream is the establishment of an adequate system of quality control. W.H.M.

- 90. Automatic ice cream packaging is here.** ANONYMOUS. Ice Cream Trade J., 43, 10: 98, 99, 164, 165. Oct., 1947.

Machines for packaging ice cream now are being manufactured by Anderson Bros. Mfg. Co., Rockford, Ill.; Pure-Pak Division, Ex-Cell-O Corp., Detroit, Mich.; Ray Industries, Los Angeles, Calif.; Frank D. Palmer, Inc., Chicago, Ill.; and Prestige Products Co., New York City.

Some of these machines already are being used commercially. Others have been fully tested and are ready for use in the plant. Still others are thoroughly tried refinements of earlier machines. All are designed to set up, fill, and seal various types of containers commonly used in the ice cream industry. All should play their part in ultimately permitting a more rapid rate of packaging with lower cost per unit and more sanitary handling.

W.H.M.

- 91. A complete report of Breyer's experiences to date with the bulk gallon.** A. L. HACKMAN, Breyer Ice Cream Co., Long Island City, N. Y. Ice Cream Trade J., 43, 10: 94, 140, 141. Oct., 1947.

This company first introduced the 1-gallon container for bulk ice cream in the Harrisburg area on Nov. 1, 1945. The first month's sales represented 3.7% of the total sales, the second month 8%, and the third month 12%, which later dropped to 4-5% of total sales. Most dealers were in rural areas. Later the gallon container was introduced in Allentown and Scranton, Pa., Wildwood, N. J., and Salisbury, Md., with sales ranging from 2 to 5.5% of total sales. Experience in the New York area was not as satisfactory, with sales averaging 2%, compared to 4 to 10% in the Philadelphia area. Apparently there is a large consumer demand for bulk ice cream in gallon containers at a reasonable price. The retail price must appear on the container. Dealers are allowed a 20% mark-up. W.H.M.

- 92. Ice cream as a nutritious food.** J. W. LAWRENCE. Can. Dairy Ice Cream J., 26, 11: 37. Nov., 1947.

Ice cream contains almost 80% dairy products, in the form of milk, cream, and milk solids. An average serving of ice cream compares favorably in mineral and protein content with apple pie, rice pudding, chocolate layer cake, lemon meringue pie, and fruit cup, and is superior to these foods in many categories. An average serving of ice cream contains only 140 calories as against 300 calories in a normal serving of apple pie, 400 in a piece of chocolate layer cake and 450 in a slice of lemon meringue pie. Ice cream is placed in the category of protective foods, containing more

proteins, calcium, vitamin A and riboflavin than many desserts. The protein and mineral content of ice cream is more easily digested than that of other sources. At retail prices it compares favorably with many other staple foods. H.P.

93. **Adding smaller markets.** ANONYMOUS. *Ice Cream Trade J.*, 43, 10: 96, 138. Oct., 1947.

Since introducing Holly Carter brand of ice cream in Milwaukee and the Crestmont brand in Detroit, the A & P stores are selling ice cream in many smaller communities. In these small markets the stores are selling ice cream purchased from local manufacturers. The usual mark-up is about 22%. No figures are available at this time on sales volume. W.H.M.

94. **Drive-in operations.** H. J. MULDOON. *Ice Cream Field*, 50, 5: 24-26. Nov., 1947.

The ideal spot for a drive-in is on the outskirts of the business district on a well-traveled thoroughfare used by local people on their way to and from the business district. For a drive-in with seating capacity of 32, a building 20' x 45' on a lot 100' x 165' with parking facilities for 40 cars is recommended.

Ice cream and products containing ice cream should be the main products sold. However, hamburgers and coffee also are essential to the best operation. Care in selecting help is stressed; young married women or divorcees with one or more children are recommended for car hops instead of boys or bobby sockers. Proper training and supervision of employees are emphasized, supervision constituting the most important phase of the whole operation. Labor and supervision costs amount to 20-22%, which is higher than for most inside stores. However, to do the same volume of business with an inside store would require about double the investment required for a drive-in. Customer advantages and complaints and operator advantages and complaints are listed. W.C.C.

95. **Planning the operation of retail drive-in ice cream stores.** O. H. GLAZIER. *Ice Cream Trade J.*, 43, 11: 66, 67, 92, 93, 94. Nov., 1947; also *Ice Cream Rev.*, 31, 5: 74, 76, 78, 80, 82. Dec., 1947.

The location should provide ample space for the parking of cars and a pleasant background. The stand should be located on the edge of the residential area rather than on a main traffic artery. There may be exceptions to this rule, such as a location that has an unusually attractive background or something of great interest near the stand that people will want to stop and see.

The building should be designed correctly, in proper proportion and in harmony with its surroundings. Some landscaping in the form of shrubs

or flower gardens helps to create the desired impression. Two dipping cabinets arranged at right angles to the front windows are desirable. One should be set at 10° F. for lower melting ice cream like chocolate and fruit ice cream and the other at 14° F. for the higher melting plain ice creams. Paper cups and dishes are best adapted to his type of business because they eliminate washing and breakage. Ample storage space should be provided. A direct expansion ammonia batch freezer is recommended, or a continuous freezer might be considered if some wholesale business is done. Storage space for cones, dishes and fountain supplies should be provided in the stand. No stand is complete without a fountain, and it should be of the proper size for the building. Mixers for milk shakes also are needed. Good wooden spoons and individually wrapped straws help to create good customer impressions.

Personnel must be chosen with care by a process of elimination. The author has had best experience with young men 18 to 25 years of age. They are faster, understand how to do things without being told all the smaller details, and do not get confused as easily in making change and in filling complicated orders during a rush.

Limit sales to ice cream and soda bar products such as sodas, sundaes and milk drinks. High-quality products sold in a friendly manner in pleasant surroundings for a fair price are the best insurance for the success of any retail ice cream store.

W.H.M.

96. Costs, prices, profits. A frank analysis of the industry's position today. V. F. HOVEY, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 43, 10: 84, 85, 145-147. Oct., 1947.

The cost of making ice cream in Sept., 1947, increased 11.5¢ per gallon over the Sept., 1946, costs, with no allowance for difference in volume. This 11.5¢ is made up of 4¢ for material, 4¢ for labor, 0.5¢ for depreciation and 3¢ for other items.

Some manufacturers may try to cut costs by increasing overrun. However, the author believes this is a mistake. A reduction in volume of sales will have a marked effect on cost. Figures indicate that a decrease of 10% in volume will result in an increase in cost of about 3¢ per gallon; a 20% decrease in volume increases the cost about 6¢ per gallon.

Since the consumer price is so closely related to consumption, manufacturers may have to print retail prices on their packages unless retailers voluntarily keep percentages of profit at a reasonable level. A sound price structure with a discount scale providing a difference in price to large and small customers, at least on bulk ice cream, equal to the difference in costs of serving them, is advocated. Manufacturers should have printed price lists to which they adhere so that each manufacturer knows the basis upon which competitors solicit business. The discounts to be given to any cus-

tomter should be determined only at the close of the year when his actual gallonage is known. W.H.M.

97. **Frozen dessert composition.** S. MUSHER. U. S. Patent 2,431,704, Dec. 2, 1947 (7 claims). Official Gaz. U. S. Pat. Office, 605, 1: 66. 1947.

As a means of controlling the overrun, avoiding bleeding and improving the texture and body of sherbets, ices and ice cream, a stabilizer consisting of oat flour is recommended. To obtain optimum results, a premix should be prepared by blending the oat flour, gelatin if used, part of the sugar and some of the water or milk of the mix and heating to at least 175° F. and preferably to 190–220° F. This mixture then is combined with the other ingredients of the mix prior to pasteurization. About 1% of the total mix should be oat flour. R.W.

98. **Ice cream and ice cream mix.** T. B. HIPPLE AND S. S. SADTLER. U. S. Patent 2,433,276, Dec. 23, 1947 (8 claims). Official Gaz. U. S. Pat. Office, 605, 4: 677. 1947.

A dry powder, suitable for making ice cream mix, is prepared by spray drying a mixture of hydrogenated butterfat, cream and soya protein. R.W.

MILK

99. **The freezing point of milk: Some recent developments** F. J. MACDONALD. Dairy Inds., 12, 9: 846–851. Sept., 1947.

The developments that have taken place since the early work of Dreser in 1892 on the freezing point of milk and its application are discussed. The average freezing point of 480 samples of milk taken from tank car shipments over a period of 2 years was -0.544° C. This figure agrees with those previously reported by most investigators.

Mastitis milk has essentially the same freezing point as normal milk, while the development of acidity increases the depression of the freezing point. The use of formalin also increases the depression of the freezing point but can be corrected for by calculation. Data from previously published work show the amount of correction required when various amounts of formalin are added. D.V.J.

100. **L'irradiation du lait aux Etats-Unis. (The irradiation of milk in the United States.)** C. WOLF. Lait, 27, 265–266: 238–257. May–June, 1947.

Irradiation methods and literature in the United States up to June, 1939, are reviewed. In a supplementary note, standards and practices adopted since that date are described. O.R.I.

PHYSIOLOGY

101. **Why hormone treatments sometimes fail.** C. F. CAIRY. J. Am. Vet. Med. Assoc., 112, 850: 30-33. Jan., 1948.

"The infancy and complexity of the hormone field make it a dangerous one for inexperienced workers. The lack of fundamental physiologic knowledge is one of the serious handicaps of hormone therapy. The exact nature of hormones and their specific action in the animal body still are unknown. Certain precautions are fundamental in any hormone therapy. Make as complete a diagnosis as possible. Evaluate environmental and clinical factors which may have same symptoms as hormone dysfunction. Use only products whose strength has been determined. Dosage is most important, since excesses often produce opposite effects. Keep up on species differences.

T.M.L.

SANITATION AND CLEANSING

102. **Some aspects of detergency involving surface chemistry and physics.** J. C. L. RESURGGAN, The British Hydrological Corp. Dairy Inds., 12, 9: 852-855. Sept., 1947.

The fundamental chemical and physical aspects of detergency are discussed. The process cannot be defined in terms of either physics or chemistry alone, since many of the physical effects are dependent upon chemical reactions in the detergent solution or at the interface between the solution and the deposit. The subject is discussed in the light of established physical laws and chemical reactions and the parts they play in the complex forces involved in the removal of deposits from metals, glass and plastics.

D.V.J.

103. **Cleansing of dairy utensils. III. Results of the bacteriological examination of rinses and swabs of farm dairy utensils.** S. B. THOMAS, P. M. HOBSON, C. G. JONES, E. JONES-EVANS, AND J. C. DAVIES. Dairy Inds., 12, 11: 1095-1099. Nov., 1947.

This investigation was undertaken to study the "rinse" and "swab" techniques of checking washed utensils on English dairy farms. Two types of farms were studied, namely, control farms using routine steam sterilization of utensils and Category C farms (600 in number) which frequently produced reject class milk.

Over 87% of rinses on control farms showed less than 50,000 bacteria, while only 31% of the Category C farms fell in this class. With swab tests, 88% of the control farms had colony counts under 5,000 per sq. ft. as compared with 27% for the Category C group. Results showed that 50% of utensils on Category C farms were grossly contaminated. The authors conclude that the swab technique is in many ways superior to the rinse method.

D.V.J.

104. Germicidal effect of quaternary ammonium compounds on dairy organisms. R. V. HUSSONG. Can. Dairy Ice Cream J., 26, 11: 92. Nov., 1947.

The advantageous properties the quaternary ammonium compounds possess for germicidal purposes are that they are soluble in water, non-corrosive, non-toxic, chemically stable, have rapid killing action, are non-volatile, are practically odorless and tasteless, are wetting agents, and have high germicidal action on test organisms used. One disadvantage of these compounds is that they are not compatible with certain alkalis and soaps used in the dairy plants. If surfaces are not rinsed free of the non-compatible materials, they may neutralize the effect of the quaternary compounds. Experimental results indicate that Roccal, Ster-Bac, Emulsept, and Isothan all were effective against *Streptococcus lactis* and *Staphylococcus aureus* and against two species of yeasts, *Torula cremoris* and *Torula sphacrica*. H.P.

105. Control of insects and rodents in food plants. G. C. DECKER. Can. Dairy Ice Cream J., 26, 11: 78. Nov., 1947.

See Abs. 134. J. Dairy Sci., 30, 4: A61. April, 1947.

MISCELLANEOUS

106. What the Federal Food and Drug is looking for. C. T. HURBLE, Minneapolis Station, Food and Drug Administration. Natl. Butter Cheese J., 39, 1: 42, 44, 46. Jan., 1948.

The Federal Food, Drug and Cosmetic Act of 1938 differs significantly from the Act of 1906 in requiring that foods be manufactured under sanitary conditions. The terms of the Act are general and are not restricted to conditions affecting the health of the consumer. Inspectors of food plants are instructed to point out failures in sanitary control to interested manufacturers. The sediment test for insoluble material and the methylene blue test for bacterial load are practical tests applied to examine the condition of milk. Penalties for violation may include seizure of the product, criminal prosecution of those responsible for violations, or injunction to cease violating the law. Fines and prison sentences may be imposed.

W.V.P.

107. One dairy plant operator influences 844 farmers. ANONYMOUS. Natl. Butter Cheese J., 39, 1: 30, 31, 64. Jan., 1948.

A survey of representative members of the dairy industry in 27 different states disclosed that 98% of them sell supplies and equipment to farmer patrons. Such sales are made to provide desirable facilities and to give fieldmen the chance to demonstrate proper methods of production. Over half of the sales are made at cost; 42% are made at regular retail prices.

Washing powders, filter discs, milk strainers, milk cans, brushes, insect sprays and sterilizers are the items most commonly handled. W.V.P.

108. Practical training for dairy technologists. C. K. JOHNS. Can. Dairy Ice Cream J., 26, 12: 21. Dec., 1947.

Theoretical knowledge is necessary but it must be supplemented by a good background of practical experience. The average college graduate was found to lack practical experience. The dairy industry must recognize that the provision of practical training is primarily its responsibility. If the dairy industry is to obtain the right type of man for positions of responsibility, some thought should be given to a program of student training. The need for well-trained men will increase from year to year. H.P.

109. West coast dairy cooperative tests key men on supervision. ANONYMOUS. Food in Canada, 7, 7: 15-18. July, 1947.

During the winter months the Okanagan Valley Cooperative Creamery Association conducts classes designed to impress foremen and supervisors as to the importance of good public relations and the necessity of careful supervision of employees. A series of 50 questions dealing with practical problems in these fields are presented in the article. Many of the questions present a number of alternative situations designed to allow the employee to appraise himself for positions of management. O.R.I.

110. New type refrigerator car requires no ice. E. M. HOLLER. Food in Canada, 7, 7: 20-22. July, 1947.

A new type of railroad refrigerator car employing an absorption ammonia system for cooling is illustrated and described. Tanks holding 1,900 lb. of liquid anhydrous ammonia are slung beneath the car. The flow of ammonia to the expansion coils in the car ceiling is controlled by bulb-type temperature control apparatus. Later the ammonia is absorbed in water in a tank slung below the car. In the test reported, the car maintained temperatures at approximately 0° F. for a 10-day trial in which the car was held in a test house at 90° F. The car was fully loaded with frozen foods and these remained in a satisfactory condition. Costs for operating this type of equipment have not been determined. O.R.I.

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

111. **Annual review of microbiology.** Vol. I. C. E. CLIFTON, editor. 404 pp. \$6.00. Annual Reviews, Inc., Stanford, Calif. 1947.

This volume initiates another series in the tradition of the "Annual Review of Biochemistry" and the "Annual Review of Physiology". Each chapter is written by one or more specialists in that particular field. The chapter on Bacterial Metabolism is reproduced from the current volume of "Annual Review of Biochemistry". Although much of the material covered is primarily of interest to specialized medical bacteriologists, the chapters on Bacterial Metabolism, Nitrogen Metabolism, Industrial Fermentations and Quaternary Ammonium Compounds contain considerable material of interest to those in the dairy industry, particularly dairy bacteriologists. Those concerned with animal health will be interested in the chapters on Antibiotics, Chemotherapeutic Agents, Immunochemistry, Some Aspects of Active Immunization and probably several others. The indexing of the book seems very satisfactory, and the numerous literature references permit the reader to consult the original publications with a minimum of difficulty.

F.E.N.

112. **Advances in enzymology.** Vol. VII. F. F. NORD, editor. 665 pp. \$8.75. Interscience Publishers, Inc., New York, N. Y. 1947.

The broad viewpoint of this volume is indicated by the fact that 5 of the 12 chapters are written by men from European countries. The approach definitely is fundamental, as each chapter covers in considerable detail the status of present knowledge in a comparatively restricted field. The chapters are titled: Permeability and Enzyme Reactions; The Properties of Protoplasm with Special Reference to the Influence of Enzymic Reactions; Recent Views on Asymmetric Synthesis and Related Processes; Some Applications of Radioactive Indicators in Turnover Studies; Bacterial Luminescence; Heme-linked Groups and Mode of Action of Some Hemoproteins; Distribution, Structure, and Properties of the Tetrapyrroles; Oxidation of Organic Sulfur in Animals; Interrelations in Microorganisms between Growth and the Metabolism of Vitamin-like Substances; Antibacterial Substances from Fungi and Green Plants; Kidney Enzymes and Essential Hypertension; and Recent Progress in Industrial Fermentation. In addition to the literature citations for each chapter, author and subject indices for this volume and cumulative author and subject indices (only for chapter titles and chapter authors) for the 7 volumes published to date are included.

F.E.N.

BACTERIOLOGY

113. **Microbiological assay for riboflavin.** H. A. KORNBURG, R. S. LANGDON, AND V. H. CHELDELIN, Dept. of Chem., Oregon State Coll., Corvallis. *Anal. Chem.*, 20, 1: 81-83. Jan., 1948.

Riboflavin is determined using *Leuconostoc mesenteroides* 10,000. The response of the organism to riboflavin permitted the development of an assay method which is sensitive to 0.0001 γ of the vitamin per ml. Good agreement is obtained among riboflavin values of samples assayed at different levels as well as good recoveries of added riboflavin. Determinations may be made turbidimetrically after 14 hr. or titrimetrically after 72 hr.

B.H.W.

BUTTER

114. **Influence of separator cleanliness-storage temperature on cream quality.** J. M. JENSEN AND A. L. BORTREE, Mich. State Coll. *Am. Butter Rev.*, 9, 5: 10, 12, 14, 16. 1947.

Cream from clean bowls held at 92 score for 72 hr. when stored at 53° F., while that from dirty bowls held a 92 score only 48 hr. When stored at 63° F., cream from the clean bowl had an average flavor score of 90.3 after 72 hr., while that from dirty bowls was maintained at 90 score for only 48 hr. Cream held at 73° F. and separated from the dirty bowl stored at 73° F. maintained a 90 flavor score for only 24 hr. Essentially as much acid was developed in 24 hr. with cream stored at 73° F. as was developed in cream stored at 53° F. for 96 hr.

Frequent delivery of cream, clean separators, and proper cooling are essential to quality cream production. Of the 3 conditions, proper cooling is by far the most important; temperatures of 53° F. or less are advisable. When cream was stored in a household refrigerator at 40° F., control of acidity development and flavor deterioration was satisfactory up to 96 hr.

P.S.L.

115. **Sediment testing of cream.** A. W. RUDNICK, Iowa State College, Ames. *Am. Butter Rev.*, 9, 12: 22, 24. 1947.

Samples for sediment testing other than those taken off the bottom of unstirred cans of cream are unsatisfactory. The following procedure has been developed to facilitate proper sediment testing of cream. Using an off-bottom tester without pad, the sample is taken from the bottom before the can is stirred. The sample then is discharged into a suitable vessel and mixed with 12 to 16 oz. of filtered water. A pad is placed in the tester and as much of the mixture drawn up as possible and discharged. The operation then is repeated with the remainder of the sample without

changing the pad. In some cases it may be necessary to dilute the sample with additional water. P.S.L.

116. **The dual use of the vacreator.** G. H. WILSTER. Oregon State Coll. Am. Butter Rev., 5, 13: 28, 30, 32. 1946.

A comparison was made at Oregon State College of butter from 86 churnings of cream from each of 2 pasteurization methods. Butter from cream pasteurized in the vacreator had an average score 0.83 point higher than butter from cream pasteurized by the vat method. Destruction of bacteria was more complete with the vacreator system of pasteurization. Similar experiments were carried on by the University of Manitoba and Iowa State College. At the University of Manitoba, in 153 comparisons, all but 8 churnings resulted in a higher flavor score when the vacreator system of pasteurization was used. Experimental results at Iowa State showed butter made from vacreator-pasteurized cream to have an average score 0.97 point higher than butter made from vat-pasteurized cream.

A study also was made at Oregon State as to the possibilities of pasteurizing ice cream mix with the vacreator. Ice cream of highly satisfactory flavor, body, texture, and melt-down characteristics was made from mix prepared by condensing the required skim milk to the proper density through the use of the vacreator, adding the other mix ingredients to the condensed skim milk, and subjecting the mixture to homogenization, vacuum pasteurization with the vacreator and cooling. The thermal efficiency of the Oregon State unit was 50.3%, but it is estimated that it could have been 85% had an efficient heat exchanger been used. The cost of removing water from skim milk was calculated to be 0.193 cent per lb. P.S.L.

117. **Cream separator.** C. E. DEARDORFF. (Assigned to C. E. Deardorff, Inc.) U. S. Patent 2,434,642, Jan. 20, 1948. Official Gaz. U. S. Pat. Office, 606. 3: 439. 1948.

A container for milk is described. It has a horizontal partition so positioned that the upper compartment thus formed is of such volume that it contains substantially all of the cream. A flap is attached to the under side of the partition which closes a hole in said partition when the container is tipped in a designated direction. The skim milk is retained in the lower compartment by the flap sealing the opening, while the cream is poured out of the upper compartment through a hole in the top. R.W.

CHEESE

118. **Manufacture of cottage cheese.** A. A. SCHOCK, S. Dak. State Coll., Brookings. Milk Dealer, 37, 2: 48, 49, 108-12. Dec., 1947.

A detailed description of the manufacture of cottage cheese by the

rennet method is given. Use of this method results in from 3 to 5 lb. more cottage cheese per 100 lb. of skim milk than is obtained by coagulating the skim milk entirely by the use of starters. The defects of cottage cheese and their causes are discussed. C.J.B.

119. **Manufacture of cottage cheese from nonfat dry milk solids.** W. H. E. REID AND M. O. MAUGHAN, Univ. of Mo., Columbia. *Milk Dealer*, 37, 1: 43, 136-138. Nov., 1947.

A new method of making cottage cheese from dry milk solids is described in detail. The method was developed by making more than 100 batches of cheese, using dry milk from 11 different manufacturers, and then checking the process on a commercial scale at 2 dairy plants. The readily available supply of spray process nonfat dry milk solids is used, enabling milk dealers to offer their customers a high-quality cottage cheese at all seasons of the year. C.J.B.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

120. **Manufacture of cultured buttermilk.** A. A. SCHOCK, S. Dak. State Coll., Brookings. *Milk Dealer*, 37, 1: 140-148. Nov., 1947.

Detailed instructions are given for the manufacture of: (a) plain or cream type buttermilk, made from skim milk or mixtures of whole milk and skim milk having a fat content of approximately 2%; (b) fat granule or fat flake-type buttermilk, which is plain type cultured buttermilk containing butterfat granules; and (c) cultured buttermilk from reconstituted skim milk. Instructions for using the creatine test for determining flavor production of cultures also are given. C.J.B.

DISEASES

121. **Research and control of mastitis in New York State.** H. C. HODGES, Vet. Coll., Ithaca, N. Y. *Milk Dealer*, 37, 1: 86-89. Nov., 1947.

Surveys made of more than 300 herds and 9,000 cows in New York State since April 1, 1946, show that the percentage of cows infected with pathogenic organisms usually associated with bovine mastitis is well over 35%, with around 25% of the cows infected showing the presence of streptococci. Hemolytic staphylococcus, coliforms, corynebacterium, and occasionally species of *Pseudomonas* also are found. Some herds have practically no infection while others show as many as 90% of the cows with mastitis organisms in their udder secretions.

Early observations indicate that control of bovine mastitis involves three closely associated phases of herd management: (a) Diagnosis, know your cows; (b) prevention, protect cows from exposure to predisposing factors and infection; (c) treatment, use judiciously after proper diagnosis. C.J.B.

122. **Scientific discussion on brucellosis at medical milk commission convention.** D. T. BERMAN, Univ. of Wis., Madison. *Certified Milk*, 22, 4: 4. Aug., 1947.

In those areas where cattle predominate in the animal industry, *Brucella abortus* is a major factor in causing human brucellosis. The test and slaughter program has gone about as far as it can go in the eradication of this disease. While calfhood vaccination is beneficial, it alone will not solve the problem; the most important thing which can be expected from calfhood vaccination in infected herds is time in which to exercise the older sanitary procedures of testing, segregation, and elimination, with the eventual aim of the development of brucellosis-free herds.

More research work is needed on the value of repeated vaccinations and also on improving the means of distinguishing infected reactors from those reacting because of vaccination with strain 19. Further research may show that it is possible to keep herds at a high level of resistance by annual vaccination. However, an important factor to consider before adopting re-vaccination or vaccination of adults is the possibility of establishing strain 19 in the udder of the lactating cow and, should this occur, its potential significance as a source of brucellosis in humans. W.S.M.

123. **Ketosis (Acetonemia) in dairy cattle.** J. C. SHAW, Univ. of Maryland, College Park. *Certified Milk*, 22, 7: 15, 16. Nov., 1947.

Ketosis, an ailment often mistaken for milk fever, is caused by a decrease in the amount of sugar in the blood rather than a decrease in blood calcium. It occurs most frequently in high-producing cows in late winter and early spring. Experimental evidence indicates: That a restricted feed intake before or after calving, or both, is probably only one of the factors causing ketosis; that ketosis is not due to a deficiency of vitamin A in the feed; and that massive doses of methionine, thiamine and other B vitamins, when administered both intravenously and orally, have no beneficial effect. The recommended treatment is the injection of glucose directly into the venous blood, supplemented by the feeding of some form of soluble sugar. When the case is difficult to diagnose or when ketosis and milk fever both are involved, it is wise to inject calcium gluconate followed by a straight glucose solution. The urine test alone is not infallible in diagnosing ketosis; it should be used in conjunction with information on the symptoms of ketosis. W.S.M.

FEEDS AND FEEDING

124. **Rumen digestion studies. I. A method of investigating chemical changes in the rumen.** E. B. HALE, C. W. DUNCAN, AND C. F. HUFFMAN. Mich. State Coll., East Lansing. *J. Nutrition*, 34, 6: 733-745. 1947.

Methods of determining digestion coefficients and rate of digestion in the rumen are discussed. The bases of calculations for such studies are treated in detail. R.K.W.

125. Rumen digestion studies. II. Studies in the chemistry of rumen digestion. E. B. HALE, C. W. DUNCAN, AND C. F. HUFFMAN. Mich. State Coll., East Lansing. J. Nutrition, 34, 6: 747-758. 1947.

Rumen digestion was studied in cows with fistulas. During the first 6 hr. following feeding, the predominant digestion was of proteins and carbohydrate. During the second 6 hr. following feeding, there was a rapid digestion of cellulose with digestion of protein and carbohydrate continuing at a rate paralleling that of cellulose digestion. Rumen digestion was complete after 12 hr. Average coefficients for rumen digestion of roughage 12 to 14 hr. following feeding were: dry matter 48.4, protein 59.6, nitrogen-free extract 65.2, crude fiber 27.2, cellulose 43.4, other carbohydrates 83.0 and lignin 3.1%. Synthesis of fatty acids in the rumen was demonstrated. Apparently there is rapid removal of the fatty acids following their synthesis. R.K.W.

126. Mineral metabolism studies in dairy cattle. I. The effect of manganese and other trace elements on the metabolism of calcium and phosphorus during early lactation. J. T. REID, K. O. PFAU, R. L. SALSBRURY, C. B. BENDER, AND G. M. WARD, N. J. Agr. Expt. Sta., SUSSEX. J. Nutrition, 34, 6: 661-676. 1947.

The effects of Ca, Mn and other mineral supplementation upon Ca and P balance were studied during the first 5 months of lactation of 12 cows. A ration of grain, corn silage, and timothy-clover hay was supplemented with either CaCO_3 , CaCO_3 plus MnSO_4 , or Mico, which was a commercial calcium source containing several trace elements. Phosphorus balances were not affected appreciably by these supplements. Manganese sulfate supplementation decreased Ca retention, while Mico increased Ca retention. R.K.W.

127. Mineral metabolism studies in dairy cattle. II. Effect of calcium and manganese and other trace elements on the metabolism of lipids during early lactation. G. M. WARD AND J. T. REID, N. J. Agr. Expt. Sta., Sussex. J. Nutrition, 35, 2: 249-255. 1948.

A study of effects of Ca, Ca plus Mn, and Ca plus Mn and other trace element supplementation upon fecal excretion of lipids was made with dairy cows. All groups of cows excreted similar proportions of total lipid intake, as measured by acid hydrolysis and chloroform extraction. However, calcium-supplemented animals were observed to eliminate lesser

proportions of ether extract. This was attributed to a greater amount of calcium soap formation in cows fed the calcium supplements, these soaps not being measured because of their insolubility in di-ethyl ether.

These data indicate the possibility of error in crude fat digestion determinations. R.K.W.

128. **Thiamine deficiency in the calf.** B. C. JOHNSON, T. S. HAMILTON, W. B. NEVENS, AND L. E. BOLEY, Univ. of Ill., Urbana. *J. Nutrition*, 35, 2: 137-145. 1948.

Thiamine deficiency in the young calf was demonstrated. This deficiency was characterized by weakness, incoordination of legs, and convulsions. In addition, some calves exhibited scouring, anorexia and dehydration. The symptoms responded to thiamine therapy except in cases of severe dehydration. R.K.W.

129. **How to make up an economical dairy ration.** ANONYMOUS. U. S. Dept. of Agr., Bureau of Dairy Industry, Publication BDIM-Inf-57. 4 pp. 1947.

A procedure based upon 4 different qualities of roughage, 3 levels of digestible protein in the concentrates and the cost per ton of total digestible nutrients in the various concentrates is outlined, and examples of the necessary calculations are given. Four tables, including one giving a schedule of suggested concentrate feeding for cows producing at different levels, are given to provide data necessary for the calculations of the most suitable ingredients under different conditions. F.E.N.

130. **More milk from your forage crops.** R. E. HODGSON, U. S. Dept. of Agr. *Certified Milk*, 22, 7: 4, 5. Nov., 1947.

When alfalfa-Ladino clover forage was made into wilted grass silage, 7.2% more dry matter and 26.4% more protein were obtained than when curing the crop as hay. The silage dry matter at the time it was fed later in the winter contained 10 times more carotene than did the hay. This difference in feed carotene showed up in the milk produced by the cows.

The advantages of the wilting method for making grass silage over other methods are: low moisture content; freedom from obnoxious odors; highly palatable, so cows will consume more dry matter; little or no drainage from silo; less expensive and troublesome to make. Harvesting experiments showed no particular difference in the amount of labor and field machinery time required to harvest a ton of dry matter as silage or as hay. Under average conditions grass silage should not be considered a substitute for corn silage but rather as a substitute for part or all of the hay and feed as such, along with corn silage. W.S.M.

131. Some observations on beef cattle affected with generalized edema or anasarca due to vitamin A deficiency. L. L. MADSEN AND I. P. EARLE, Bureau of Animal Ind., Beltsville. *J. Nutrition*, 34, 6: 603-619. 1947.

During the period of July, 1941, to Dec., 1946, 651 beef carcasses were condemned for generalized edema or anasarca by Federal meat inspectors. This condition was observed to occur in cattle after a long period in dry lot when the diet consisted of corn and a low-carotene roughage, such as oat hay or straw.

Cases of anasarca were produced experimentally by feeding carotene-deficient rations. Alfalfa hay was effective in curing the condition in the field. Blood studies also showed similarity between experimental and field cases of anasarca; such condition apparently is due to vitamin A deficiency. R.K.W.

132. Comparison of vitamin A liver storage following administration of vitamin A in oily and aqueous media. A. E. SOBEL, M. SHERMAN, JACQUELINE LICHTBLAU, SELIG SNOW, AND B. KRAMER. Jewish Hospital of Brooklyn, N. Y. *J. Nutrition*, 35, 2: 225-238. 1948.

Liver storage, in rats, of vitamin A from oily and aqueous media was studied. Vitamin A liver storage was greater when the vitamin A source was dispersed in water than when vitamin A was in the oily medium. These data indicate importance of considering the nature of diluent for biological evaluation of vitamin A. R.K.W.

FOOD VALUE OF DAIRY PRODUCTS

133. Nutritional studies on milk fat. III. The effect of the treatment on milk fat with certain solvents on the growth of young rats. E. L. JACK AND E. B. HINSHAW, Univ. of Calif., Davis. *J. Nutrition*, 34, 6: 715-724. 1947.

Pentane (Skelly-solve A) contains an impurity which lowers the growth-promoting properties of milk fat. Treating the pentane with fuming sulfuric acid removes this deleterious action. A -53° filtrate milk-fat fraction prepared with the purified pentane produced greater growth in young rats than the original fat. Previously a -53° fraction milk fat, obtained by using unpurified pentane, gave slightly less growth than untreated milk fat. R.K.W.

ICE CREAM

134. Control of raw materials and partly finished products in the ice cream plant. A. H. BAYER, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 43, 12: 44. Dec., 1947.

The use of daily factory records is desirable and makes possible the control of profits affected by production. These records are current, and they point out inefficiencies in operation, possible theft, waste in labor and operation, and other conditions that could not otherwise be observed. Factory records account daily for materials and products used, for materials and products sold and for those remaining in inventory. Simple forms worked out by the production and accounting departments will make possible the collection of needed information in a neat, orderly and uniform manner. Standards for each operation, such as units of production per man hour in the mixing, freezing, packaging, novelty and other operations, may be set up.

A list of the factory records which may be used includes: a daily report of mix made, a monthly mix report, a daily freezing report, a daily report of operations, a withdrawal slip for charging out products from the hardening room, a report showing products put into the hardening room, a hardening room inventory report, and a planning report showing ingredients needed, finished product desired, hours and people needed for the job.

Records properly kept will provide information on cost of products, proper selling prices, proper planning of production and efficient plant operation, point out possible theft, and aid in quality control. They are a necessary part of any manufacturer's business. W.H.M.

135. Evaluating the flavor of ice cream. D. V. JOSEPHSON, Dept. of Dairy Technol., Ohio State Univ., Columbus. *Ice Cream Trade J.*, 43, 12:48. Dec., 1947.

Attempts were made to determine consumer preferences with respect to the sugar, butterfat and serum solids content of ice cream. Groups of students tested over a period of 3 yr. indicated that 75% consistently preferred ice cream containing 14 to 15% sugar, 60% preferred ice cream containing 12% fat, and the percentage preference increased for serum solids as the amount was increased from 8 to 13%.

In the evaluation of flavor, three functions are used: (a) taste (gustatory response), which is the result of certain organs on the tongue; (b) touch (tactual response), which is the result of the stimulation of other organs on the tongue; and (c) smell (olfactory response), which comes from the chemical stimulation of the olfactory cells located at the base of the nasal cavities. People do not have the same degree of development or refinement in their taste functions. Other complications are involved, such as temperature of samples, size of sample, eating habits of the individual, age and rate of recovery of taste mechanism between samples. In most judging work a panel of 3 to 5 judges is used. This system has certain weaknesses, such as time of judging, number of samples tasted,

presence of a dominant figure who sets standards for the group, and wide range of numerical points assigned to flavor scores on all factors, which cause results from the panel to vary from the results which might be obtained from an average consumer.

Suggestions are offered for improving present methods of evaluating flavor of ice cream. The relative sensitivities to basic, abnormal and deteriorative flavor qualities of each member of the panel should be determined. If numerical ratings are used, a maximum range of 5 or 6 points should be employed. Each taste observer should have complete freedom in expressing his judgment. A standard and uniform terminology for describing the flavor and texture qualities of ice cream should be established. Judging sessions should be planned so that observers will have ample time, preferably before meals. Avoid disturbance or noise. Evaluate the product in terms of consumer preference, if known. Remove all identification marks from samples. The individual who sets up the samples and takes the data should not be a member of the panel. W.H.M.

136. Utilizing the true lactic acid content to indicate ice cream quality.

I. A. GOULD AND F. A. POTTER, Univ. of Maryland, College Park.
Ice Cream Trade J., 43, 12: 46. Dec., 1947.

Some method other than the titration procedure is needed for the determination of the actual lactic acid content of ice cream. In recent years the Hillig colorimetric method for the determination of lactic acid has been developed in the laboratories of the Pure Food, Drug, and Cosmetic Administration and has been used to indicate the quality in dry milk solids; the procedure has been accepted by the Association of Official Agricultural Chemists. The Hillig method was applied to ice cream.

The method used was essentially the A.O.A.C. method with modifications. Of 10 samples of ice cream tested, 6 contained less than 13 mg. % of lactic acid. No relationship was found between the lactic acid content and titratable acidity and pH of the mixes. The method was found to be accurate generally within 2 mg.%. Normal values were increased somewhat by addition of certain flavoring components but not sufficiently to invalidate the use of the lactic acid method as a quality test. W.H.M.

137. The use of liquid sugar in ice cream. H. G. DUNLAP, H. P. Hood and Sons, Inc., Providence, R. I. Ice Cream Trade J., 43, 12: 54. Dec., 1947.

The Hood Co. has been using liquid sugar for 17 yr.; economy and quality problems were responsible for the practice. A saving of 30 cents per cwt. in purchase price and 15 cents net in handling costs has resulted. No trouble was experienced with fermentation; however, it was found necessary to keep the Brix at 67° in order to prevent crystallization. Sirup

is trucked as far as 100 miles and rail tank cars are used for shipping 240 miles to a condensery. Storage is not much of a problem but requires planning on the estimated amount needed. Meters and an auxiliary 100-gallon tank are used to measure the sirup; however, the meters are not as satisfactory as the calibrated tank. W.H.M.

- 138. The Bowman dairy program for building "dry stop" volume.** H. A. QUITTER, Bowman Dairy Co., Chicago, Ill. *Ice Cream Trade J.*, 43, 12: 42. Dec., 1947.

A dry stop setup for improving the appearance of dealers' stores and increasing profits has been developed. The setup consists of a 5-ft. tile board back bar, with a 4-ft. fluorescent light behind a canopy. In the center of the back bar is a permanent flavor board and on either side 2 frames in which advertising may be displayed. Electric connections for mixers and hot cups are provided. This bar is hung on the wall about 30 in. to the rear of the ice cream cabinet and a counter is fitted on top of the cabinet. A specially constructed sirup tray holding 4 2-qt. shallow pans is fitted into one of the openings of a wooden flip-lid cabinet. A dipper well also is connected to the cabinet. Through an arrangement with a paper cup company, the ice cream dealer is furnished with a combination package consisting of 300 4-oz. cups for a 10 cent sundae, 300 8-oz. cups for a 20 cent sundae, 300 16-oz. malt cups, 300 banana split boats, and 2 metal mixing collars for mixing malts and milk shakes in the 16-oz. cups.

The salesman is paid a bonus of 1.5 cents per gallon for every gallon each dealer increased over the previous year's business and a penalty of 2 cents for every gallon under the previous year's business.

In attracting people to ice cream stores, animated displays are to be preferred over the paper window displays. The fountain is placed in the brightest spot in the store and pictures of the various items sold are displayed at the fountains. Trained, neat, courteous, and efficient help is employed. Cleanliness at the fountain, good quality toppings and uniform servings help increase fountain business. W.H.M.

MILK

- 139. Proper store differentials.** R. C. CRABB, General Ice Cream Corp., Schenectady, N. Y. *Milk Dealer*, 37, 2: 42, 80-82. Dec., 1947.

Based on experiences in 4 markets in upper New York State and in New England, the author points out that a grocery store owner today is realizing the same penny profit per quart of milk as he did years ago when milk sold for several cents a quart less. Many grocers treat milk as a poor relation and do not realize that milk can pull more customers into their stores than any other item, with the possible exception of bread. Some un-

favorable factors involved in milk handling are: the low profit per unit of sale, the refrigeration required, the handling of empty bottles and the supplying of a bag. However, milk has the first or second highest turnover of any item in the store. Being perishable, milk attracts customers to the store daily. Surveys show that 86% of people returning bottles make purchases averaging 66 cents. No money is tied up in inventory, as with canned goods. No space is needed for storage of extra inventory either in the basement, back room, or in an outside rented warehouse.

The milk dealer should aid the grocer to increase his milk sales by urging him to keep his milk displayed, preferably in a self-service case, and by using sales promotional material in his store. The dollar sales should be built up so that the per cent of profit will be of secondary importance. Through a process of continually talking in terms of dollars and cents rather than per cent of profit, the grocer's apparent indifference to milk sales can be broken down. C.J.B.

140. **Fat variation in milk tests.** T. M. BINNEY, Purdue Univ., Lafayette, Ind. *Am. Milk Rev.*, 8, 8: 52-53. 1946.

Factors which may be influential in causing variation in fat tests are: breed of cow, individuality of cows, feed, season, weather, stage of lactation, age, time interval between milkings, completeness of milking, health of the cows, exercise, management, environment, farm skimming practices, and conditions during transportation of the milk. During the month of May composite herd tests from one herd varied from 4.7 to 5.8%; daily variations on composite samples ranged from 0 to 0.7% fat. In another herd, during the same month, fat tests from composite herd samples ranged from 5.1 to 5.9% and daily variations ranged from 0 to 0.6% fat. P.S.L.

141. **Consideration on the keeping quality of pasteurized milk.** L. H. BURGWALD AND D. V. JOSEPHSON. Ohio State Univ. *Am. Milk Rev.*, 8, 1: 26-47. 1946.

Regular grades of pasteurized milk were found to stay sweet for 7 to 28 days under conditions which might normally be imposed upon it by consumers. Milk was considered sweet until the acidity increased by 0.03%. The average keeping quality under all conditions studied was greater than 12 days. The initial bacterial counts did not always indicate potential keeping quality of the milk. On the average, the low count milk had very slightly better keeping quality than the higher count milk. There was no appreciable increase in bacterial count for the first 4 days. After 4 days, the psychrophilic bacteria increased rapidly and invariably numbered over a hundred million at the time the milk soured. No flavor change was noted in any of the samples before 4 days. The half pints held below 40° F. developed a flavor change at an average of 12.1 days.

The average vitamin C content was 10.5 mg. per l. in the fresh milk which had not been unduly exposed to light or room temperature. The quarts exposed for 2 hr. at room temperature contained 8.5 mg. per l. after exposure. The vitamin C in 4 lots had been reduced from 7.4 to 0.6 mg. per l. in 2 days even without exposure, probably due to iron or copper contamination. The milk from the other 4 lots not exposed had an average of 13.7 mg. of vitamin C per l. in fresh milk, 9.7 mg. at 2 days, and 6.4 mg. at 4 days. The riboflavin content of all lots of milk was practically the same, with an average of 1.57 γ per ml. Practically none was lost upon storage, the average being 1.55 γ per ml. at the point of souring. P.S.L.

142. A test for the milk plant as an added protection. A. V. MOORE AND G. M. TROUT. *Am. Milk Rev.*, 8, 8: 22-24. 1946.

Five series of homogenized and non-homogenized milk to which various increments of raw milk were added were observed for the development of rancidity, changes in titratable acidity and pH, and reaction to the phosphatase test. At a storage temperature of 40° F., rancidity developed in samples of homogenized milk which were contaminated with raw milk, and the titratable acidity increased, along with a decrease in pH. Detection of raw milk contamination was shown by the phosphatase test when 0.5% of raw milk was present but was questionable when only 0.1% of raw milk was present. It was found necessary to have at least 4% of raw milk present to detect contamination in homogenized samples at the end of 24 hr. upon the basis of rancidity as judged by taste and smell. Extraction of the phenol with butyl alcohol was found necessary in order to make the results of the phosphatase test accurate for homogenized milk.

P.S.L.

143. Technical considerations in leveling milk production. L. C. CUNNINGHAM, Cornell Univ. *Am. Milk Rev.*, 8, 9: 48-54. 1946.

Factors in leveling fall milk production include consumer demand for milk, seasonality of milk prices received by farmers, and dairy farm organization and income. Seasonality of milk production is increasing in importance due to an increase in consumer demand for fluid milk. In New York state, the cost of producing milk is 30% lower in the summer and 25% higher in winter than the average cost for the year.

To facilitate the study of seasonal patterns in milk production, dairy farms in each of 7 areas in New York were classified as even dairies, spring dairies, summer dairies, and winter dairies. Even dairies included those in which daily deliveries in the lower quarter were at least 70% of deliveries in the higher quarter. Spring dairies had their highest quarter in April-June, summer dairies in July-Sept., and winter dairies in Jan.-March. Farm records used were not all for the same period. Herds

tended to be larger in the even dairies, but generally there was little relationship between seasonal production and herd size.

Production per cow definitely was higher in the winter dairies than in spring dairies, the average difference ranging from 600 to 2,000 lb. per cow, depending on the area. Production in summer dairies was the lowest of all types. The labor force is used more efficiently in winter dairies. The output of milk per man in winter dairies exceeded that found in spring dairies in all areas; in 3 particular areas the amount was 0.5 can daily per man. The year-round cost of producing 100 lb. of milk was lower in winter dairies than in spring dairies, the difference ranging from 6 to 20% in various areas. The average difference in cost was 30 cents per cwt.

Winter dairies were more profitable than spring dairies, the difference in income ranging from \$300 to \$1,200, depending upon total income. Differences between income from winter and even dairies were insignificant. Summer dairies were the least profitable. The relatively higher incomes in winter and even dairies were due primarily to higher milk production per cow and per man and to a considerably lesser extent to higher milk prices in the fall. An increase in fall and winter milk production to meet consumer demand is in line with sound dairy farm management.

P.S.L.

144. Paper bottle. F. D. PALMER. (Assigned to F. D. Palmer, Inc.) U. S. Patent 2,435,155, Jan. 27, 1948. Official Gaz. U. S. Pat. Office, 606, 4: 642. 1948.

A rectangular-shaped paper container is described suitable for distributing milk and other liquids. For dispensing the product in a sanitary manner, an opening and pouring spout arrangement is provided which is sealed at time of filling and which cannot again be resealed once it is opened.

R.W.

145. Refrigeration for the milk bottling plant. L. BUEHLER, JR., Creamery Package Manufacturing Co., Chicago, Ill. Milk Dealer, 37, 1: 92-98. Nov., 1947.

A refrigerating plant must be of sufficient size, reliable in operation, require a minimum of attention, and be simple enough that the operator need not be highly skilled. The advantages and disadvantages of the direct expansion system in which the refrigerant, such as ammonia, is used directly in the milk cooler, ice cream freezer or room cooling coils to extract the heat from the product and the chilled water system, with and without ice storage, are discussed. The choice would depend upon the peculiar requirements of any specific job.

C.J.B.

- 146. Sales, expenses, and profits of six leading milk companies in the New York-New Jersey Metropolitan Area.** L. SPENCER, Cornell Univ., Ithaca, N. Y. *Milk Dealer*, 37, 1: 45, 46, 117-122. Nov., 1947.

During the 6 yr., 1941-1946, 6 of the principal companies distributing milk in the New York-New Jersey Metropolitan Area sold \$159 million worth of products yearly, their net profit amounting to 1 cent per dollar of sales. These companies had smaller profits per dollar of sales and per dollar invested by the owners than any of a number of representative groups of companies in different kinds of business throughout the country. Companies manufacturing drugs and soap averaged 8.4 cents per dollar of sales; 15 dairy companies doing business in various parts of the country, including operations in ice cream, evaporated milk and cheese, earned 2.5 cents per dollar of sales in this 6-yr. period.

The return on the owners' investments in the 6 milk companies for the period was 3.3%. This rate was lower than that for any of 17 important groups of companies whose financial statements are made public. During the period studied the dollar sales of the 6 milk companies increased 67% while the amount paid for milk and other products purchased went up 87%. These gains were due mainly to the general advance in prices rather than to growth in physical volume of business. Product cost amounted to 55% of sales in 1941 and 61% in the year ending June 30, 1947, while the gross spread between sales and product cost became 44% greater.

A little more than 12 cents a quart is paid by the dealer for his milk supply at country receiving plants. Operating costs of all kinds on milk delivered to the consumer's doorstep come to nearly 10 cents; of this amount, more than 6 cents goes for delivery service. The dealer's profit is perhaps as much as 0.5 cent a quart before taxes are paid. This is equivalent to 1.5 cents per dollar sales. Of the 20 cents a quart paid by consumers for a quart bottle of standard milk at stores, 2 cents is retained by the storekeeper and 18 cents goes to the milk dealer. His operating costs total about 5.6 cents, including 2.4 cents for delivery service. Under present conditions his net profit after all taxes is no more than 0.2 cent a quart, or 1 cent per dollar of sales. C.J.B.

SANITATION AND CLEANSING

- 147. A comparison of the cleaning of square and round milk bottles under regular commercial conditions.** T. V. ARMSTRONG AND L. H. BURGWARD. Ohio State Univ. *Am. Milk Rev.*, 8, 1: 34-37. 1946.

Washed bottles were obtained from 7 different makes of washers in 9 different plants during the months from June through Sept., 1945. Some of the washers were able to accommodate the square bottle without any

change, while others had to undergo adjustments. The dirty bottles used were the regular run of bottles as returned to the plant or brought from another plant. The procedures used in making the counts were those outlined in the Eighth Edition of "Standard Methods for the Examination of Dairy Products". After plating, the bottles were examined under a daylight lamp and the presence of specks or streakiness was noted. The bottles then were filled with milk and again examined for specks and streaks.

Counts in excess of 1,000 per bottle were obtained on 4.3% of the square quart bottles and 7.6% of the round bottles. Specks of dirt were found in 1.1% of the square bottles and 5.3% of the round bottles. Of 15 different lots of one-half pint bottles, counts in excess of 250 per bottle were obtained on 9.6% of the square bottles and on 8.6% of the round ones. No visible dirt was observed in any of the square bottles of this size, while visible dirt was observed in 8.6% of the round ones. These studies and data show that there is no difference in the commercial practicability of cleansing and sterilizing the returnable square milk bottles and the conventional round bottle in typical dairy soaker equipment.

P.S.L.

MISCELLANEOUS

148. **Waste saving and disposal.** O. W. SONBORG AND H. J. STEFFEN, Chicago, Ill. *Am. Butter Rev.*, 9, 7: 40-43. 1947.

Dairy wastes can be reduced by drip savers on can washers, steaming cream cans, pre-rinse of milk cans, electronic liquid level controls on vats, accurate temperature control on plate coolers, standby power equipment, adequate storage tanks, prevention of leakage, prevention of foaming, saving of buttermilk for livestock feed, and provision of facilities for processing skim milk. Some waste from rinsings and wash water is unavoidable. When the amount of waste is small, a septic tank may be sufficient as a means of disposal. Another elementary treatment is accomplished through the use of an aerated equalizing tank. Trickling filters may be used to give more complete treatment when necessary. The filters are composed of a coarse medium, such as gravel or coke, and the sewage allowed to flow over this medium at such a rate as to allow free passage of air.

P.S.L.

149. **The training of young men for the dairy industry.** R. B. STOLTZ, Dept. of Dairy Technol., Ohio State Univ. *Milk Dealer*, 37, 2: 86-90. Dec., 1947.

Thirty-five years ago the universities and colleges were not attempting to turn out dairy technologists or managers for dairy plants; they were training buttermakers, ice cream makers, and other dairymen—who knew how to produce milk and manufacture it into dairy products. Today the attempt is to train executives whose foundation will rest upon chemistry,

mathematics, physics, bacteriology and accounting. The Ohio State curriculum today does not require students to take agricultural subjects such as entomology, farm crops, soils, horticulture and geology. Instead, they are required to take applied subjects, such as speech, marketing, advertising and salesmanship.

The author also discusses the training of students after they have finished the university. An employer should take the men when they are available and not wait until he needs them. Too much should not be expected of the graduates, as they still are boys. The men should be contacted to demonstrate management knows they exist, given responsibility, encouraged to make progress, given a change of work, shown that management has confidence in them, and should be paid what they are worth. C.J.B.

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ABSTRACTS OF LITERATURE

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ABSTRACTS OF LITERATURE

BOOK REVIEW

150. **Legal aspects of milk sanitation.** JAMES A. TOBEY. 133 pp. \$5.00. Milk Industry Foundation, Washington, D. C. 1947.

This is the second edition of a book dealing with the legal responsibilities of milk producers and processors and distributors of milk and milk products. It is concerned with responsibilities relating to public health rather than to price fixing. The sources and nature of laws and regulations relating to milk sanitation are described. Many court decisions and opinions are cited which show the validity of various laws and regulations. The book is of value as a source of information concerning the extent and limitations of responsibilities of handlers of dairy products insofar as these products are related to public health. Chapter headings include the following: I. Reasons for the Public Control of Milk, II. The Sanitary Regulation of Milk by the State, III. Municipal Control of Milk, IV. Licenses and Permits, V. Standards for Milk and Milk Products, VI. Inspection, Sanitation and Seizure of Milk, VII. Tuberculin Testing and the Health of Dairy Cattle, VIII. Pasteurization, IX. Containers for Milk, and X. Liability in Connection with Dairy Products. M.P.B.

BUTTER

151. **Controlling the composition of butter.** R. W. BROWN. Can. Dairy Ice Cream J., part 1, 26, 11: 33. Nov., 1947; part 2, 26, 12: 23. Dec., 1947.

The composition of butter is controlled because it must conform to state and federal standards, operating losses must be reduced to a minimum, and butter must retain its weight and moisture to avoid danger of short weighting. A knowledge of the factors that affect overrun and overrun control is necessary in order to control the composition of butter. Calculations are given for overrun, theoretical overrun, composition overrun, churn overrun, creamery overrun, accuracy of testing cream, losses of fat in buttermilk, overrun of unsalted butter, and water addition. H.P.

152. **Die Bedeutung des Acetoin und Diacetyls für das Aroma von Rahmsäurungskulturen.** (The importance of acetoin and of diacetyl upon the aroma of butter cultures.) J. RODENKIRCHEN. Die Milchwissenschaft, 2, 8: 329-335. Aug., 1947.

Butter cultures grown in heat-treated skim milk (75° C. for 30 min.) and held for 3 days varied in intensity of the acetoin-diacetyl reaction when examined daily. The intensity of the acetoin-diacetyl reaction did not correlate with presence or absence of chains of organisms or with the

quality of flavor produced. Generally the intensity of the reaction of the individual cultures remained at the same level for 3 days when cultures were incubated at 20° C. for 20 hr., followed by a lower holding temperature.

The acetoin and diacetyl formation was found to be independent of the pH of the starter cultures. Also, the amount of these compounds formed was not influenced by the available oxygen supply.

Higher incubation temperatures (50–60° C.) increased the production of acetoin and of diacetyl. Reheating of the cultures resulted in a more distinct acetoin-diacetyl reaction. Growth of butter cultures in whole milk generally resulted in higher production of acetoin and diacetyl than growth of the same cultures in skim milk. The author states that the acetoin-diacetyl reaction according to Vas Criszár is not suitable for objective measurements of the quality of flavor in butter cultures. I.P.

CHEESE

153. **A rapid method for determining extraneous matter in Cheddar cheese.** E. G. HOOD. *Can. Dairy Ice Cream J.*, 27, 1: 19–20. Jan., 1948.

A 15-g. sample of Cheddar cheese is placed in a clean Waring Blendor jar of 1,000-ml. capacity, and 200 ml. of 10% sodium citrate solution at a temperature of 75 to 80° C. is added. After 2 minutes of disintegration, the sample is transferred to a funnel under suction, using a lintine filter disc. The advantages over the older method for removing the extraneous matter from Cheddar cheese are: (a) a smaller sample can be used (15 g. compared to 227 g.); (b) hot citrate solution is used for more rapid disintegration; (c) the sample can be obtained from one cheese plug, preventing damage to the cheese; and (d) the method is much faster. The test can be carried out in 4 minutes, an added incentive for extending the use of the method to an educational program or to regulatory control. H.P.

154. **Apparatus for use in the centrifugal separation of serum from cheese constituents.** G. J. STREZYNSKI. (Assigned to DeLaval Separator Co.) U. S. Patent 2,436,498, Feb. 24, 1948 (12 claims). *Official Gaz. U. S. Pat. Office*, 607, 4: 683. 1948.

The details are given for a continuous type separator bowl which may be used for removing serum from a standardized coagulated dairy product, thus producing a curd of such moisture content that it may be used for cream cheese. R.W.

CHEMISTRY

155. **Lactoflavin-reduktase.** (Riboflavin-reductase.) M. E. SCHULZ. *Die Milchwissenschaft*, 2, 3: 152–160. March, 1947.

The role of riboflavin as an oxidation-reduction indicator in milk and

its relative position with respect to other redox systems are pointed out. The usefulness of riboflavin as an indicator of oxygen tension in growing cultures of *S. lactis* in milk is demonstrated and the role of riboflavin in the formation of diacetyl in cream ripening is discussed. I.P.

156. **The use of the sodium-chlorine relationship for the detection of sodium neutralized non-fat dry milk solids.** W. HORWITZ, Food and Drug Administration, Federal Security Agency, Minneapolis, Minn. J. Assoc. Offic. Agr. Chemists, 31, 1: 121-124. 1948.

The sodium and chlorine contents of 23 samples of sodium-neutralized and 81 samples of normal or non-sodium-neutralized dry skim milks were determined. The ratio of sodium to chlorine in the normal samples was 0.47, with a standard deviation of 0.03. If the per cent sodium is plotted as the abscissa against the per cent chlorine as the ordinate, all of the sodium-neutralized samples lie to the right of the line represented by the equation: $\% \text{ Na} = 0.62\% \text{ Cl} - 0.10$. All of the normal or non-sodium-neutralized samples lie to the left of this line. F.J.B.

157. **Serum methods for added water in milk.** D. J. MITCHELL AND G. G. FRARY, State Chemical Lab., Vermillion, S. D. J. Assoc. Offic. Agr. Chemists, 31, 1: 124-127. 1948.

The mean value of 3 serum methods (acetic serum, sour serum, and copper serum) did not indicate added water until more than 10% added water was present. The copper serum method was the most rapid from the standpoint of preparation of the serum and gave a narrow range of readings. The authors state that the cryoscopic method should be used to check the serum methods whenever added water is indicated, since the method is rapid, accurate and most reliable. F.J.B.

158. **The determination and identification of lactic and succinic acids in foods.** H. V. CLABORN AND W. I. PATTERSON, Food and Drug Administration, Federal Security Agency, Washington, D. C. J. Assoc. Offic. Agr. Chemists, 31, 1: 134-139. 1948.

A method is outlined for the determination of lactic acid in liquid whole or skim milks and in dried whole or skim milks. Detailed procedures are given for preparation of the sample, preparation of the sodium salt of lactic acid, preparation of the partition column, isolation and identification of lactic acid. F.J.B.

159. **The determination of free tryptophane in milk, cream and butter.** R. E. DUGGAN, Food and Drug Administration, Federal Security Agency, New Orleans, La. J. Assoc. Offic. Agr. Chemists, 31, 1: 151-162. 1948.

A method is described for the extraction and measurement of the free

tryptophane in milk, cream and butter. Investigations show that negligible quantities of free tryptophane are present in normal sweet cream and milk. The amount of free tryptophane in milk and cream increases with age if the products are held under conditions conducive to bacterial and enzymatic activity. The amount of free tryptophane in butter depends upon the free tryptophane content of the original cream. F.J.B.

160. **Residual chlorine in milk after the addition of hypochlorite.** F. B. MORELAND, Kansas State Board of Health, Topeka. J. Assoc. Offic. Agr. Chemists, 30, 4: 655. 1947.

Sodium hypochlorite was added to milk in concentrations ranging from 1,000 to 5 p.p.m. and the milk allowed to stand at room temperature for varying periods of time. At certain intervals, the amount of residual chlorine was determined by titration with thiosulfate and by the Rupp test (A.O.A.C. Methods of Analysis, 6th ed., 1945, p. 317). Data show that the residual chlorine of the milk decreased rapidly. At a chlorine dosage of 100 p.p.m. in the milk, the value dropped to zero in about 15 min. However, the Rupp test continued positive long after the residual chlorine reached zero. The author states that the term "available chlorine" as used in the heading of the table interpreting the reactions to the Rupp test is apt to be misleading, since the values given are for the amount of available chlorine which is present at the instant of its addition to the milk rather than when the Rupp test actually is performed, perhaps much later. Tests also were made on milk containing 100 to 5 p.p.m. available chlorine and held in a refrigerator. F.J.B.

161. **Das Fettverderben und seine Bedeutung für Wirtschaft und Leben.** (The deterioration of fat and its importance in economy and in life.) H. SCHMALFUSS. Die Milchwissenschaft, 2, 8: 335-347. Aug. 1947.

The review covers 726 publications and patents issued between 1893 and 1944, dealing with fat deterioration. The author distinguishes between 5 main types of fat deterioration, namely, tallowness, peroxide formation, hydrolysis, ketone formation and aldehyde formation. I.P.

162. **Biophysical studies of blood plasma proteins. VIII. Separation and properties of the gamma globulins of the sera of normal cows.** E. L. HESS AND H. F. DEUTSCH, Univ. of Wis. J. Am. Chem. Soc., 70, 1: 84-88. 1948.

The conditions of separation, yield and some physical properties of normal bovine serum gamma globulins are reported. The procedure recovers 85% of the gamma globulins, which can be further separated into fractions with varying electrophoretic mobilities. As a result of this and

previous studies, it is anticipated that practical and economical ethanol fractionation methods for the removal of antibody from hyperimmune sera soon will be practical.

H.J.P.

- 163. Crystalline pepsin-resistant protein from skeletal muscle.** J. BOURDILLON, N. Y. State Dept. of Health, Albany. Arch. Biochem., 16, 1: 61-68. 1948.

A crystalline protein-like substance characterized by its high resistance to peptic hydrolysis was isolated from beef skeletal muscle. It represents at least 2% of the total proteins and is different from known fractions of muscle. Its physiologic role is unknown. The term *peptomyosin* is proposed for this substance. A similar fraction has been extracted from horse skeletal muscle.

H.J.P.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

- 164. Report on sampling and analysis of condensed buttermilk.** RAGNAR E. BERGMAN, State Dept. of Agr., St. Paul, Minn. J. Assoc. Offic. Agr. Chemists, 30, 4: 613. 1947.

The total solids content of a sample of condensed buttermilk was determined by drying at 100° C. under vacuum, drying at 100° C. without vacuum, drying at 70° C. with vacuum, drying at 70° C. without vacuum, and by the toluene distillation method. The average results obtained by 10 collaborators using the above methods were 27.71, 27.21, 29.59, 29.26, and 29.98%, respectively. When the same methods were used, but with 2 g. of recently ignited zinc oxide and 5 ml. of water added, the results obtained by the first 4 methods were 28.67, 28.04, 29.19 and 29.22%, respectively; the toluene distillation method was not used with this procedure. The use of zinc oxide tended to prevent charring of samples. It was recommended that collaborative work on sampling and analysis of condensed buttermilk be continued.

F.J.B.

DISEASES

- 165. The clinical diagnosis and treatment of breeding unsoundness in cows.** J. W. CUNKELMAN. J. Am. Vet. Med. Assoc., 112, 852: 292-295. April, 1948.

Failure in conception is not always the fault of the female. A large percentage of diagnosed conception failures can be treated successfully.

T.M.L.

FEEDS AND FEEDING

- 166. The value of supplementary vitamins for calves raised under artificial conditions.** A. A. SPIELMAN, D. B. H. DALRYMPLE, C. L. NOR-

TON, J. K. LOOSLI, AND K. L. TURK. *Am J. Vet. Research*, 9, 30: 26-29. Jan., 1948.

Results of a large-scale field trial to determine the value of supplementary vitamins for calves raised under good, fair and poor farm conditions of feeding and management are reported. In the first trial, alternate calves received daily, from birth to 30 days of age, a tablet of 5,000 I.U. of vitamin A, 250 mg. vitamin C, 1,000 USP units vitamin D, and 100 mg. niacin. The other calves received a placebo capsule. In experiment 2 the dosages of vitamins were increased. The feeding of extra vitamins to newborn calves, raised under farm conditions of good, fair and poor feeding and management, did not materially reduce the incidence or duration of scours or result in any apparent improvement in the general appearance and condition of the calves up to 30 days of age. T.M.L.

167. **Vitamin A and trace minerals in the diet of dairy cattle.** P. E. NEWMAN. *Cornell Vet.*, 28, 1: 69-78. Jan., 1948.

The need for adequate vitamin A in the diets of dairy animals becomes more apparent each year. One common source of vitamin A deficiency is in young calves which have not received enough milk for necessary needs; the other is in cows which are pregnant and need an extra amount of vitamins above that found in the average ration.

Cobalt deficiency can be easily corrected by feeding cobalt sulfate in salt at a rate of 1 oz. to 100 lb. Over-feeding of this mineral is dangerous. No general regions for iron deficiency have been observed. Copper is deficient in Florida. Several Great Lakes States and a few others are deficient in iodine. Although manganese is needed by dairy cattle, no clear-cut deficiency areas have been observed. Present information does not indicate a likelihood of a natural deficiency of boron, zinc or fluorine. Caution should be exercised in the supplementing of trace minerals due to the lack of information on exact needs. T.M.L.

ICE CREAM

168. **Basic ice cream industry stabilizers.** C. D. DAHLE AND W. F. COLLINS, Pa. State College, State College. *Ice Cream Field*, 50, 6: 24, 25, 26, 35. Dec., 1947.

With a mix containing 12% fat, 11% serum solids and 15% sugar, a perfect texture score was obtained with ice cream having 0.5% gelatin 150 Bloom, 0.42% gelatin 200 Bloom, and 0.35% gelatin 250 Bloom. The pH of gelatin did not affect the results. Dariloid (sodium alginate) was found to give a perfect texture in the above mix when 0.275% was used. Sodium alginate from another source gave comparable results when used in concentration of 0.15%. Locust bean gum (carob) is not satisfactory

as a sole stabilizer because of excessive wheying off of the mix; 0.25% is required for stabilization. Locust bean gum works very well for ices and sherbets and is most important for hot pack cream cheese.

Dried extract of Irish Moss commonly is used to prevent sedimentation in chocolate milk drinks. As little as 0.08% will provide for good mix stabilization but will give high initial viscosities and contribute to a permanent foam. It commonly is used in mixed proprietary stabilizers. Sodium carboxy methyl cellulose, commonly known as CMC or cellulose gum, is tasteless and odorless. When used to the extent of 0.15 to 0.18% in ice cream mixes, it gives good stabilization and results in low viscosity mixes. Slight separation may occur in the mix after storage for about 2 weeks at 35 to 40° F. Pectin commonly is used in ices and sherbets. It was found that 0.15 to 0.25% of 150 grade pectin in ice cream did not properly stabilize the product, although no separation occurred in the mix, which is contrary to previous claims. Ground psyllium seed husks used to the extent of 0.15% gave ice cream with perfect body and texture. Unless a coarse mesh strainer was used ahead of the homogenizer, a considerable amount of the stabilizer was removed. This stabilizer gives a low viscosity mix. Karaya gum long has been used in ices and sherbets and has been employed in mixed stabilizers. About 0.35% is required to stabilize an ice cream mix, but it was found that the smooth texture was of short duration.

The authors report that all of the stabilizers gave satisfactory overrun and that there were not objections to melt down characteristics if the proper amounts were used. In dipping trials with ice creams stabilized with gelatin, Dariloid, CMC and dried extract of Irish Moss, there was no difference in the amount of ice cream dipped from a 2.5-gallon can when the ice cream was tempered to 6° F.

W.C.C.

169. **Outlook for cream and milk solids in 1948.** J. M. PUNDERSON, Rochester Dairy Coop., Rochester, Minn. *Ice Cream Rev.*, 31, 6: 52, 71. Jan., 1948.

Plenty of butterfat and milk solids for the manufacture of ice cream will be available in 1948, but the price for these products is not expected to drop as low as it did in 1947. Strong domestic consumer demand for fluid milk and cream, export demand, low carry-over stocks of dairy products, and unfavorable milk production trends are cited as the reasons for believing that neither butterfat nor milk solids will show any significant price decline during 1948.

W.J.C.

170. **Ice cream mix.** A. LEVITON. (Dedicated to the people of the U.S.) U. S. Patent 2,433,850, Jan. 6, 1948 (4 claims). *Official Gaz. U. S. Pat. Office*, 606, 1: 74. 1948.

It is claimed that in an ice cream which is subject to sandiness, the

addition of 3.5 mg. of riboflavin per 100 g. of water will cause the lactose to crystallize in thin trapezoidal plates, a form which does not produce the suggestion of sandiness in the mouth. Other dairy products, such as sweetened condensed milk, also may be made free of lactose graininess through the addition of riboflavin. R.W.

171. Controlling labor costs in retail outlets. ANONYMOUS. *Ice Cream Rev.*, 31, 6: 42-44, 110. Jan., 1948.

A practical procedure which has been used successfully by one company to meet the challenge of increasing labor costs in the operation of retail stores is presented. Determination of sales by hours is accomplished by hourly checks of the cash register for a week every month for each store with an operating staff of 5 or more persons. The per cent of gross sales can be allowed for labor cost and thus the total amount of money which can be spent daily for labor is determined. This in turn may be figured on the basis of sales per man hour as a labor efficiency guide.

The next step is to determine how the daily man hours should be distributed by hours of the day to provide adequate help for all phases of store operation. Once this has been established the next job is to work out the labor shifts so the proper number of employees will be present when needed. Help for part-time shifts usually can be provided by ladies in the neighborhood who desire part-time employment.

The final step is to prepare a master list of shifts which describes each shift in terms of duties and numbers of hours of daily work. The master list of employee shifts provides a convenient simple method for telling old and new employees what days they work, the hours they are on duty and when they will have their days off.

The charts accompanying this article should prove valuable to any manager of a retail store who wishes to analyze the labor setup for his particular store or group of stores. It is reported that one firm operating 50 fountains in San Francisco was able to reduce store operating hours from two shifts to one in approximately 50% of its locations because of excessive labor costs during lean hours of the day. W.J.C.

172. Billboard advertising. ANONYMOUS. *Ice Cream Rev.*, 31, 6: 45. Jan., 1948.

Members of the International Association of Ice Cream Manufacturers now may obtain at cost from their association billboard posters for use in an outdoor advertising program to supplement their regular advertising activities. These posters are attractive in design and feature top-notch promotional ideas to stimulate ice cream sales. A place for the imprint of the individual firm is provided so the billboard becomes an effective promotional activity of the firm making use of this service. W.J.C.

MILK

- 173. Program for improving milk quality.** E. M. BARKER, Rochester Dairies, Rochester, Minn. *Milk Dealer*, 37, 4: 102-112. Jan., 1948.

The following broad fundamental or basic principles are listed as essential in a successful quality improvement program: (a) The management of a particular dairy enterprise must be sold on the merits of an improvement program and willing to allocate a substantial sum of money each year over a period of years for its promotion. This naturally involves the development and continued utilization of markets which will pay for quality products. (b) Through such a program economic benefits must accrue over the years to the participating producers. (c) The establishment of differentials and incentives is necessary, based on grades which will accomplish the specific objectives desired. Milk quality will improve more rapidly and will be maintained at a desired level. (d) The recognition that mere regulation will not perform the rightful task of the particular institution and producer is essential. Both management and producers must not fail to recognize that together they must develop and maintain a satisfactory program.

In addition, all producers must use the methods and equipment necessary to continuously supply high quality milk. The milk from such producers must be uniformly and regularly graded according to rigid, prescribed standards. All information pertaining to the milk supply of the producer must be passed on as rapidly as possible to him. A field force, properly directed and made up of well-trained, practical-minded men, is necessary. Platform testing under the supervision of trained intake men is a continuous task. Cans and can washers must be maintained in a satisfactory and sanitary condition. A constant program of education and service to producers must be in effect at all times. C.J.B.

- 174. More economical system of homogenizing milk.** J. V. QUIGLEY, Chapman Dairy Co., Kansas City, Mo., AND W. A. CORDES, Sealtest, Inc., New York, N. Y. *Milk Dealer*, 37, 4: 41, 42, 116-124. Jan., 1948.

A new process of homogenizing milk, in which milk is separated and only the cream homogenized, is revealed. The cream going to the homogenizer has a fat content of 8 to 9%. Trials have indicated that satisfactory results will be obtained when the test of the cream does not exceed 13.0%. This process, used with a short-time, high-temperature pasteurizing unit in a closed system, has been in successful commercial operation since Feb., 1945. It has reduced the time of operation of the plant on homogenized milk to about one-third of the time required for the homo-

genization of whole milk, thus resulting in economies involving power, steam, light, refrigeration and labor. Homogenized milk produced by the new process has been demonstrated to be a satisfactory product as judged by top and bottom test differentials, curd tension, microscopic appearance and sedimentation due to leucocytes. C.J.B.

175. **Delivery problems relating to the single service container.** D. DEAN, *Dean's Dairy*, Champaign, Ill. *Milk Dealer*, 37, 4: 46, 100. Jan., 1948.

The delivery problems relating to the single service container are divided into the two distinct categories of long distance hauling and local wholesale and retail delivery. The company has found that the larger units of the tractor trailer type are the most practical for long distance hauling. Smaller units, or so-called "straight jobs" designed to carry 5 tons or less, are too small for a long haul, resulting in overloading of the motor, tires, etc., and the possibility of running afoul of the existing local and state highway regulations. Smaller trucks also are economically unsound because the pay load is not large enough to cover the cost of a union driver whose wages are governed by a union scale covering the larger inter-state trucking companies. In local delivery the type of truck required is essentially the same as that used in delivering milk in glass bottles. It is possible to haul a much greater pay load on a local wholesale or retail truck when single service containers are used. Elaborate plans for refrigeration are not necessary, although ice may be used as a precaution in extremely hot weather. Trucks should be insulated as much as possible, with partitions back of the driver. C.J.B.

176. **How to lose money in the milk business.** C. F. ROSEBRUGH. *Can. Dairy Ice Cream J.*, 27, 1: 34-42. Jan., 1948.

The tangible ways to lose money in the milk business are through processing and bottled goods shrinkages, ticket and bottle discrepancies, and credit losses. The intangible losses include price cutting, waste plant capacity, waste vehicle capacity, waste manpower capacity, lack of organization, lack of accounting information, and lack of uniform industry costs. H.P.

177. **Milk can production in Austria.** ING. OTTO WOLFRUM, Vienna, Austria. *Milk Dealer*, 37, 4: 74-80. Jan., 1948.

The milk cans produced in Austria are described in full and comparisons made with those produced in the United States. Photographs are used for illustration. C.J.B.

- 178. Portable milk pasteurizing apparatus.** E. F. MANGOLD. (One half assigned to H. P. Chapman.) U. S. Patent 2,436,585, Feb. 24, 1948 (8 claims). Official Gaz. U. S. Pat. Office, 607, 4: 704. 1948.

An ordinary 10-gallon can of milk is placed on a platform in a portable container. A pump operated by a motor circulates water from the bottom of the container through a heater and sprays it around the neck of the milk can. The same motor operates a small propeller which keeps the milk agitated. R.W.

- 179. Fiberboard cream separating milk container.** C. E. DEARDORFF. (Assigned to C. E. Deardorff, Inc.) U. S. Patent 2,436,140, Feb. 17, 1948 (6 claims). Official Gaz. U. S. Pat. Office, 607, 3: 494. 1948.

A paper milk bottle is described which contains a horizontal partition at about the place where the cream line forms. As the milk creams in the container, the cream collects in the upper compartment, from which it may be removed by pouring. During the pouring process the skim milk is retained in the lower section as the result of the V-shaped edge of one side of the dividing partition. R.W.

- 180. Separator.** K. S. WRISLEY. U. S. Patent 2,436,029, Feb. 17, 1948 (2 claims). Official Gaz. U. S. Pat. Office, 607, 3: 467. 1948.

A V-shaped tubular siphon-type separator for removing cream from bottles of creamed milk has for its chief novel feature a telescopic arrangement which permits adjusting the opening to any desired level within the bottle. R.W.

- 181. Milk bottle cap.** H. W. BUDAN. U. S. Patent 2,434,787, Jan. 20, 1948 (2 claims). Official Gaz. U. S. Pat. Office, 606, 3: 476. 1948.

A cap or closure for glass milk bottles is described which is designed to be used by the consumer from the time the milk bottle is opened until its contents is finally consumed. It may be made of metal or other material sufficiently stiff, yet elastic, that it will lock itself to the top of the bottle by a skirt or projections provided for the purpose. An integral part of the device is a hook which easily and cleanly removes the customary paper board disc cap used to close glass milk bottles. R.W.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

- 182. Industry builder. The biography of Chester Earl Gray.** · ROBERT E. JONES. 233 pp. Pacific Books, P. O. Box 558, Palo Alto, Calif.

This work is a history of the Dry Milk Institute as well as an interesting biography of the life of Chester Earl Gray, giving his family background and portraying the American way of life. In it are listed most of the names of men who have contributed toward the progress of the dairy industry, particularly in the dry milk branch, during the past generation. Anyone interested in the progress of the dairy industry should have a copy of this work.

R.B.S.

BACTERIOLOGY

- 183. A study of variability in duplicate standard plate counts as applied to milk.** J. L. COURTNEY, Milk and Water Laboratory, Oak Ridge Department of Health, Oak Ridge, Tenn. Milk Plant Monthly, 36, 12: 22-24, 27-28, 30. 1947.

A standard plate count may be duplicated by the same laboratory with an average variation of less than 20%. Some variations are surprisingly small. Milk samples were plated experimentally by each of 4 methods. The human error often extends the actual variation in plate counts greatly beyond their proper proportions, while the result seemingly is the normal variation of the method employed. Consciousness of the abuse of the standard plating procedure and a definite effort to improve the technic employed will make the standard plate count a more valuable tool in control programs.

G.M.T.

- 184. Improved techniques for the microscopic analysis of milk.** G. H. WATROUS, JR., Pa. State Coll. Milk Plant Monthly, 36, 10: 42-43. 1947.

Details are given for the strip-counting technique for the microscopic analysis of milk, as well as for suggested stain and staining techniques. The authors find that less variability between operators and less fatigue on the part of technicians examining large numbers of samples result when prescribed procedures are used.

G.M.T.

185. Psychrophiles, mesophiles, thermophiles, and thermodurics—what are we talking about? J. C. OLSON, JR., Univ. of Minn. Milk Plant Monthly, 36, 11: 32–36. 1947.

Mesophilic, thermophilic, thermoduric and psychrophilic bacteria are terms used for moderate heat-loving, heat-loving, heat-enduring and cold-loving groups of bacteria, respectively. There are no well-defined temperature zones for each of these classes of bacteria. Each group may present a special problem and many require a different method of control. Sixteen references are cited.

G.M.T.

186. The significance of certain bacteria in pasteurized milk. M. L. SPECK, N. C. State Coll., Raleigh, N. C. Milk Plant Monthly, 37, 2: 36–38, 43. 1948.

Bacteria which have significance regarding sanitary practices employed during the processing of pasteurized milk are, mainly, thermoduric micrococci, thermophilic bacteria, coliform bacteria, and microbacteria. The occurrence of any group in large numbers in milk should be interpreted as a warning that more serious trouble in the form of milk-borne diseases or loss of consumers from the sale of unpalatable milk may result if immediate steps are not taken to correct the faulty practices which permit them to be present.

G.M.T.

187. The action of penicillin in-vitro on organisms found in bovine mastitis. H. F. FARRAG. J. Am. Vet. Med. Assoc., 112, 854: 371–374. May, 1948.

A number of organisms commonly found in milk were incubated with cultures containing various concentrations of penicillin. Micrococci were found to be highly sensitive to penicillin action and were fairly uniform in susceptibility. Different strains of *Streptococcus agalactiae* varied significantly in susceptibility to penicillin, while strains of *Staphylococcus aureus*, isolated from the same source, were much more uniform in sensitivity. In one case of streptococcal mastitis milk which was heavily contaminated with *Escherichia coli*, 50% of the penicillin was destroyed when incubated with the milk filtrate for 3 hr. It is suggested that the action of the enzyme, penicillinase (thought to be produced by *Escherichia coli*), may explain some of the variable results from treatment with penicillin.

T.M.L.

BUTTER

188. Butter defects—their causes and prevention. V. J. BEIMBLECOMBE. Australian J. Dairy Technol., 3, 1: 36–39. 1948.

The causes and remedies of a large number of defects are given in

brief form in this resume of an address. Flavor and aroma defects, body and texture defects and color defects are included. F.E.N.

CHEESE

189. **Making Gouda cheese in Queensland.** W. J. PARK. Australian J. Dairy Technol., 3, 1: 34-36. 1948.

In this abstract of an address, quite complete directions for the manufacture of Gouda cheese are given and the causes of defects and the means of prevention also are discussed. Of particular interest is the procedure for salting the curd before hooping. A report of the discussion of the address is included. F.E.N.

CHEMISTRY

190. **On the Babcock test for fat in dairy products.** L. R. SCHARP, D. I. SHEW, G. LOFTUS HILLS, R. TREMBATH, AND H. R. WEBB. Australian J. Dairy Technol., 3, 1: 15-23. 1948.

This is a report of a sub-committee of the Victorian Division of the Society of Dairy Technology. The specifications on apparatus and methods given in British Standards Institute Pub. 755, parts I and II, were preferred to the A.O.A.C. methods. The 8% bottle rather than the 10% one was preferred, with the accuracy of calibration within $\pm 0.05\%$. The BSI standards for pipette calibration and operation were favored; the top of the meniscus should be used for measuring the milk sample. Specific gravity of the acid should be standardized to ± 0.002 instead of the ± 0.005 commonly used. Addition of acid in one lot was approved. The A.O.A.C. specifications for tester speed and temperature were preferred. Standardized lighting for reading tests is advocated. The average reading of the Babcock test was considered 0.04% higher than by the R  se-Gottlieb ether extraction method. Reducing the quantity of milk delivered by the pipette to 17.82 g. was the procedure preferred for correcting this difference; reading all milk tests to the nearest 0.1% below the actual reading was the correction of choice until a change in pipette specifications was adopted universally. Brief recommendations for testing cream also are given.

F.E.N.

191. **Configuration of vaccenic acid.** P. C. RAO AND B. F. DAUBERT. Dept. of Chem., Univ. of Pittsburgh. J. Am. Chem. Soc., 70, 3: 1102-1104. March, 1948.

The infrared pattern of vaccenic acid (isolated from beef tallow according to Bertram) was compared with oleic acid and elaidic acid; the *trans* configuration of vaccenic acid is confirmed. H.J.P.

192. **Studies on lactoglobulins.** J. A. BAIN AND H. F. DEUTSCH. Dept. of Chem. & Physical Chem., Univ. of Wisconsin. *Arch. Biochem.*, 16, 2: 221-229. Feb., 1948.

Fractionation procedures of both bovine and goat lactoglobulins by means of alcohol precipitation methods (Cohn) are described. Lactoglobulins showing one peak upon electrophoresis at a given pH were obtained. More than one component, however, was revealed when pH mobility curves were determined. On sedimentation analysis, both preparations showed one component, but only the bovine lactoglobulin appeared to be molecularly homogeneous. H.J.P.

193. **The thermodynamics of metallo-protein combinations.** Copper with bovine serum albumin. I. M. KLOTZ AND H. G. CURME. Chem. Lab., Northwestern Univ., Evanston, Ill. *J. Am. Chem. Soc.*, 70, 3: 939-943. March, 1948.

The extent of binding of cupric ions by bovine serum albumin was measured by the equilibrium dialysis technique at pH 4.8 and 0 and 25° C. Free energies, entropies and enthalpies have been calculated for the multiple equilibria involved. The cation-protein linkage is through the carboxyl group; a stable complex can be formed when the carboxyl group is in suitable juxtaposition with other substituents or residues. H.J.P.

194. **The binding of some sulfonamides by bovine serum albumin.** I. M. KLOTZ AND F. M. WALKER, Chemical Laboratory, Northwestern Univ., Evanston, Ill. *J. Am. Chem. Soc.*, 70, 3: 943-946. March, 1948.

The formation of complexes between 6 sulfonamides and crystallized bovine serum albumin was investigated. The energy of binding data has been correlated with structural features of the drugs. H.J.P.

195. **Ionic exchangers in the dairy industry.** O. F. GARRETT, M and R Dietic Laboratories, Inc., Columbus, Ohio. *Milk Dealer*, 37, 6: 50, 132-140. March, 1948.

The ionic exchange process is explained by describing the zeolite process of treating hard water. When hard water is passed through a bed of zeolite, the calcium is removed from the water and held by the zeolite; at the same time sodium is given up by the zeolite to the water, thereby converting the scale-forming calcium salts to non-scale-forming sodium salts. The total mineral content of the water remains virtually unchanged. The end result is soft water. The cationic and the anionic types of exchangers are described.

The following applications of this new basic process to dairy products are outlined: The preparation of soft curd milk; the preparation of soluble sodium caseinate; the treatment of wheys, whereby essentially 100% of the lactose present in the original whey is recovered; the preparation of dried cream products containing from 50 to 70% butterfat which reconstitute well in water, are perfectly stable in hot coffee, and do not curdle when used with acid fruits or in making creamed soups; and the stabilization of evaporated milk.

C.J.B.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

196. **Milk and milk products in bread making.** F. R. DAVIDSON. Australian J. Dairy Technol., 3, 1: 40-42. 1948.

The addition of lard or a similar fat at the time ordinary skim milk powder is added to the dough mix or the use of a milk powder containing the fat which was emulsified into the milk before drying is reported to overcome the volume defects associated with the use of 6% milk powder in bread. This amount of powder is considered necessary to increase properly the nutritive value of bread. In addition to the resume of the address, a report of the discussion which followed also is given.

F.E.N.

197. **Aeration of butterfat containing liquids.** C. A. GETZ. (Assigned to Aeration Processes, Inc.) U. S. Patent 2,435,682, Feb. 10, 1948 (6 claims). Official Gaz. U. S. Pat. Office, 607, 2: 280. 1948.

Whipped cream is produced by aerating the cream in a variety of ways with nitrous oxide gas, with or without such other gases as cyclopropane, dimethyl oxide, methyl chloride or diflouridichlormethane. It is claimed these gases permit the use of cream containing 30% fat or lower, the use of homogenized cream and unaged cream. The whipped cream exhibits no clumping of fat globules, the serum drainage is minimized and bacterial growth inhibited.

R.W. .

198. **Factors to consider in making high quality chocolate milk drinks.** W. C. THACKER, Farmers' Cooperative Dairy, Winston-Salem, N. C. Milk Plant Monthly, 36, 1: 30-32. 1948.

Six desirable characteristics of chocolate milk are: (a) Use of high quality milk or part-skimmed milk, (b) mild chocolate flavor, (c) little or no sedimentation accompanied by low viscosity, (d) elimination of ragged, off-colored cream layer, (e) light to medium red color, and (f) medium to high sweetness. The amount of chocolate used in chocolate milk ranges from 1 to 1.5% of the finished product. When liquid chocolate is used, the amount ranges from 1.50 to 2.25%. Sedimentation is minimized by eliminating in-

soluble particles, increasing fineness of grinding, increasing the viscosity of the product, and decreasing the difference in density between the suspended particle and the suspending medium. Homogenization does not prevent settling of cocoa in chocolate milk. Stabilizers vary in their ability to prevent sedimentation. Creaming can be prevented by pasteurizing the milk at 160–165° F. for 15–30 min. A 5% sugar is suggested, while an 8% sugar is about the maximum sweetness tolerated. Production abuses often arise when processors do not follow the specific directions accompanying chocolate sirups. G.M.T.

- 199. Evaluation of procedures for the manufacture of cottage cheese.** W. I. TRETSVEN, Dairy Advisory Service, Chicago, Ill. *Milk Plant Monthly*, 36, 11: 28–30, 70, 72. 1948.

Much of the cottage cheese today is made from pasteurized skim milk. The previous heat treatment given the skim milk may determine the amount of heat required to cook the curds. Among the known factors which affect the amount of heat required to cook cottage cheese are the acidity of the curd and whey, the concentration of the curd-forming constituents, the size of the curds, the amount of agitation, the types of acids present and the presence of gas. Some commercial practices involve gas formation, which heretofore has been discriminated against. Under certain prescribed conditions gassy curds may be cooked economically and the curds firmed rather quickly. G.M.T.

- 200. The manufacture of cottage cheese.** A. A. SCHOCK. S. Dak. State Coll. *Milk Plant Monthly*, 36, 10: 82–86. 1947.

The manufacture of cottage cheese involves: (a) pasteurizing the milk at 143° F. for 30 min.; (b) setting the milk at 86° F. by adding 5 lb. of starter for 100 lb. of milk and the addition of 1 ml. of rennet per 1,000 lb. of skim milk, plus 2 oz. of 50% calcium chloride per 1,000 lb. of skim milk if formation of desirable coagulum shows this addition necessary; (c) cutting the curd at a whey acidity between 0.45 and 0.55%; (d) cooking the curd slowly to a temperature yielding a desired firmness of curd; (e) draining and washing the curd with cold water within a 0.5-hr. period; and (f) creaming so that the fat content is approximately 4% and salting at the rate of 1 to 1.5 lb. per 100 lb. of cottage cheese. The defects in cottage cheese manufacture are slow setting, tough and rubbery curd, and soft and pasty curd. Remedies are suggested for these defects. G.M.T.

- 201. Preparation of kefir fermented milk.** L. A. BURKEY, Bureau of Dairying, U. S. Dept. of Agr. *Milk Plant Monthly*, 37, 1: 48–49. 1948.

Kefir fermented milks are acid milks which have been fermented by

means of kefir grains. These may be obtained in dry form from the American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C. The kefir grains may be perpetuated by transferring them every 2 or 3 days into a new supply of skim or whole milk by rinsing them in clean cold running water before transfer. The kefir milk may be prepared by heating the milk to 165° F., cooling to 70° F., suspending the kefir grains at the rate of 1 part to 3 parts of milk and incubating at 70° F. Large quantities of kefir buttermilk may be obtained by suspending the kefir grains in cheese-cloth bags at the surface of the milk. When sufficient acidity (about 1%) has been developed, the grains may be removed and the milk agitated to produce uniform smoothness, then cooled and bottled. G.M.T.

202. **The manufacture of cultured buttermilk.** A. A. SCHOCK, S. DAK. State Coll. Milk Plant Monthly, 36, 10: 48-50, 52, 54. 1947.

See Abs. 120, J. Dairy Sci., 31, 4: A46. April, 1948.

FOOD VALUE OF DAIRY PRODUCTS

203. **Studies on the comparative nutritive value of fats. X. On the reputed growth-promoting activity of vaccenic acid.** H. J. DEUEL, JR., S. M. GREENBERG, EVELYN E. STRAUB, DOROTHY JUE, C. M. GOODING, AND C. F. BROWN. Dept. of Biochemistry and Nutrition, Univ. of Southern Calif., Los Angeles, and Bayonne Laboratory, The Best Foods, Inc. J. Nutrition, 36, 3: 301-314. March, 1948.

When the diet of rats contained either cottonseed oil or butterfat, no difference in rate of growth was found. When the diet contained rape-seed oil, growth was less. Decreased efficiency of utilization of the rape-seed oil was attributed to its high content of eracic acid. Vaccenic acid or hydrogenated China wood oil fed to the rats on the rape-seed oil diet did not increase growth, nor did vaccenic acid fed to rats receiving the cottonseed oil diet. R.K.W.

ICE CREAM

204. **Evaluation of vanilla.** J. MERORY. Ice Cream Rev., 31, 8: 54, 56, 60, 62. March, 1948.

Manufacturers and chemical users of vanilla flavor must learn to use their senses of smell and taste in the evaluation of vanilla. Results of laboratory analysis are of value in protecting the consumer against fraud, but they do not in themselves indicate the quality of vanilla flavor. Basic factors which determine the quality of vanilla flavor include quality of vanilla bean used, care given the bean before and after extraction, method

of extraction, quality of menstrum, procedure for blending and aging of the vanilla extracts, and type of container used during aging.

Vanilla flavor is obtained by extraction only, and not more than a 4-fold concentration of vanilla is possible by extraction. Distillation is to be avoided, since vanilla flavor is difficult to evaporate and the process tends to destroy the fragrance of the aromatic compounds of the vanilla beans.

Manufacturers of vanilla extracts should insist that in the purchase of vanilla beans the complete nomenclature of origin, grade, size, date of cure, and moisture content be shown on the label of the container in which they are packed. The chemical reaction which produces vanillin and other aromatic compounds in the bean continues as long as the moisture content remains unchanged. Storage of beans, therefore, under suitable conditions for a prolonged period of time prior to extraction is deemed advisable.

Typical analytical values are given for vanillin, total ash, lead number, alkalinity of total ash, soluble ash, color and resin of vanilla extracts, and applications of these values are discussed.

W.J.C.

205. Average butterfat content in ice cream 12% during 1947. ANONYMOUS. *Ice Cream Trade J.*, 44, 3: 52. March, 1948.

The average butterfat content of ice cream in 1947 was 12% compared to 10.3–10.6 during 1944 and 1945, when government controls were in effect. This figure is considerably above the minimum butterfat requirement in many states. Five states have an 8% minimum, 23 states a 10% minimum, 14 states a 12% minimum, 1 state a 13% minimum, 4 states a 14% minimum, and one state has no standard.

W.H.M.

206. High temperature-short time pasteurization of ice cream mix. C. M. MINTHORN, Chester Dairy Supply Co., Chester, Pa. *Milk Plant Monthly*, 37, 2: 70–73. 1948. Also *Ice Cream Rev.*, 31, 8: 45, 97, 98, 100. 1948

Ice cream mix may be pasteurized successfully by high temperature-short time pasteurization. Usually a higher temperature than the standard 160° F. for 15 seconds is employed. The temperature recommended is 176° F. for 22 seconds. The author concludes: (a) Ice cream mix can be heated without burn-on continuously for long periods of time. (b) Heating of ice cream mix containing frozen fat from storage temperature to 125° F. in less than 1 min. prevents oxidation of the fat. (c) High temperature-short time pasteurization decreases the amount of equipment, with a saving of time and labor. (d) When mix is prepared in a condensing pan, high preheating temperatures can be used for increased efficiency of the condensing equipment and, even more important, for stabilizing milk solids.

G.M.T.

207. Controlling processing operations. J. C. NESMITH, Southern Dairies, Inc. *Ice Cream Rev.*, 31, 7: 54, 56. Feb., 1948.

In the control of labor costs in the ice cream plant, 4 factors of major importance are discussed. (a) New employees should be advised in writing of all company rules and policies so there will be no chance for misunderstandings developing at a later date. It is suggested that the company in turn should keep a permanent and complete file on each employee. (b) A weekly work schedule setting forth the kind and amount of each product to be manufactured should be planned in advance. In addition, a daily work schedule should be prepared which will show the hours each employee is to be on duty and what the nature of his duties will be. (c) Overtime pay should be eliminated. To accomplish this, have employees dress for work before punching the time clock; allow a staggered 15-min. rest period each morning and afternoon; have the timekeeper check all pay cards a day or two before the close of each week's operation to determine if time is in line with the normal work week—if not, an adjustment in work schedule may be necessary. (d) Records should be kept so that the actual labor cost on each and every product may be determined. The detailed information for such records can best be obtained by the department foremen who, in turn, will give the information over to the accounting department. The accounting department will then be able to supply the management with accurate labor-cost figures on each item manufactured. W.J.C.

208. Full utilization of the plant laboratory. H. McAULIFFE, Bowman Dairy Co., Chicago, Ill. *Ice Cream Trade J.*, 44, 3: 56, 89-93. March, 1948.

Most ice cream manufacturers recognize the need for a laboratory but fail to utilize one properly. Considerable time can be saved by deciding what information is desired and necessary. Decisions on how samples should be taken, who should take them and how they should be cared for are just as important as deciding what samples should be taken.

The effectiveness of the laboratory often is lost because of lack of authority or failure to exercise its authority to follow through and take steps to prevent the recurrence of faulty practices. The laboratory should be used not only to check dairy ingredients, but its activities should be expanded to include examination of non-dairy ingredients and, in some instances, paints, fuels and other materials purchased. Developmental research on modification of the product can be handled with the control program. The laboratory can be used to give some technical training to new employees. It also can be used as a part of a public relations program. W.H.M.

209. Shrinkage. H. A. BENDIXEN, State College of Washington, Pullman. *Ice Cream Trade J.*, 44, 2: 46, 47, 90-94. Feb., 1948.

The following precautions are suggested for guarding against shrinkage:

(a) Avoid freezing the ice cream too stiff in the continuous freezer, since under such conditions the air in the ice cream might be present under considerable pressure. (b) Avoid extreme temperature changes or heat shocking of the ice cream, especially if a high overrun is obtained. If ice cream is hardened with dry ice or in very low-temperature freezing tunnels, do not change the ice cream suddenly to high temperatures. A more gradual change is preferable. (c) Use high-quality, low-acid dairy products to prevent destabilization of the proteins. (d) Avoid an excessively high sugar content and especially a high dextrose content, which would increase the fluidity of the ice cream at any given temperature of storage. (e) Avoid the use of unparaffined cartons or cans, banging of the packages, and excessive air circulation directly over the ice cream in the storage room.

W.H.M.

210. **Present status of ice cream shrinkage.** J. A. LEEDER, Ramsey Laboratories, Cleveland, Ohio. *Ice Cream Rev.*, 31, 8: 152. March, 1948.

Suitable temperature control of ice cream during freezing, hardening and storage is regarded as the most important factor involved in the shrinkage problem. The following recommendations are made with respect to temperature control as a means of guarding against shrinkage: Avoid freezing ice cream excessively stiff and dry at the freezer. Maintain ice cream hardening rooms at -10 to -20° F., with distributing branches at -5° F. Avoid temperatures below -20° F. Maintain uniform temperatures in all hardening and storage rooms. Do not load trucks with insufficiently hardened ice cream. Transport trucks should be maintained at below 0° F. and retail trucks at -2 to $+4^{\circ}$ F. Avoid exposure of ice cream to dry ice in trucks or in cabinets. Maintain retail cabinets at -15° F. or below.

Other factors mentioned which may cause shrinkage or produce ice cream susceptible to shrinkage are: Use of sweetened condensed milks; use of excessive amounts of dextrose or other sweeteners which contain a high mineral content or which are high in acidity; use of stabilizers containing an emulsifier such as polyhydric alcohol ester or the use of egg yolk; high overrun; incorporation of air pockets into cans or containers as they are filled; use of paper containers which are not properly paraffined on the inside surface.

W.J.C.

211. **The retail store, past—present—future. Part 2.** D. GHORMLEY. *Ice Cream Trade J.*, 44, 3: 46-48, 93-96. March, 1948.

The development of the retail store from 1935 to 1942 is discussed. Expenses, merchandising, standardization of products and location are among the factors important for successful operation.

W.H.M.

212. **Sherbets, the status of.** ANONYMOUS. *Ice Cream Trade J.*, 44, 3: 50, 101-102. March, 1948.

The production of 12,520,000 gallons of sherbet in 1947 was down 18% from 1946 and 81% below the record 1945 production according to statistics issued by the U.S.D.A. The volume of sherbets made in 1947 was still 50% greater than that of the last pre-war year, 1941. It appears that sherbets have returned to the minor status that they occupied during the pre-war years in the over-all industry picture.

W.H.M.

213. **The new cabinet look.** ANONYMOUS. *Ice Cream Field*, 51, 1: 30, 32, 34. Jan., 1948.

The merchandising program of Sealtest, National Dairy Products Corp., New York, built around the "Ice Cream Mart" is described. The mart consists of an 8-hole ice cream cabinet, enhanced by a front of baked enamel and a counter of stainless steel. Provision is made for display of attractive advertising material. Ten points are listed upon which the merchandising program is based. Ice cream is an impulse-buying item; the mart is for the exclusive sale of ice cream; strict cleanliness should be observed in the use of the mart. Since April, 1947, about 4,000 sales marts have been installed by Sealtest dealers in principal towns and cities of 33 states.

W.C.C.

214. **The efficient use of cabinet and truck space.** L. C. ANDERSON, General Ice Cream Corp., Schenectady, N. Y. *Ice Cream Trade J.*, 44, 1: 42-43, 68-70. Jan., 1948.

The problem of how much cabinet space to furnish, how many deliveries to make, and the size of the trucks which need be used is one that cannot be made the subject of some mathematical formulae but must be studied from the standpoint of individual markets and individual customers within such markets. A study should be made to secure data as to the proper size of cabinet to furnish a customer, the proper number of days to make deliveries and the day of the week on which to make deliveries. Such a study would make it possible to establish a sound cabinet and delivery policy and eliminate excessive cabinet holes and excessive number of deliveries. The determination of the proper delivery days will assure the dealer ample stock and maximum sales.

W.H.M.

215. **Stimulating volume through maximum use of cabinets.** W. D. DOBSON, Carnation Co., Los Angeles, Calif. *Ice Cream Trade J.*, 44, 3: 54-55, 99-101. March, 1948.

Information on how to build sales volume through better use of cabinets,

reducing costs through optimum use of equipment, and the cost of furnishing cabinets and cabinet rental schedules is presented. In dry stops, the cabinet should be where it can be seen and where there is heavy store traffic; near the check stand usually is a good location. A lighted super structure, a tilted mirror showing the inside of the cabinet, and attractively arranged stock are points which have been used effectively by chain stores. In wet stops, visual display of packaged and bulk ice cream has been found to increase sales. The display of related items such as cones, cakes, cookies and toppings may be used to attract attention and sell these items, as well as ice cream, in increased amounts.

The right-sized cabinet is needed for the various accounts; studies should be made at frequent intervals to determine if the cabinets are being utilized effectively. Cost figures should be determined on cabinets of various sizes. For example, this company has determined that a 12-hole, double-row conventional cabinet costs \$10.34 per month, consisting of: depreciation (6 yr.), \$3.94; interest (6% on the unamortized balance), \$1.06; field labor servicing cabinets, \$1.06; maintenance and material, \$0.31; shop repair and labor, \$1.62; and overhead in cabinet department, \$2.35. W.H.M.

216. A study of ice cream delivery practices. ANONYMOUS. Ice Cream Trade J., 44, 2: 44, 45, 85. Feb., 1948.

Information is presented on frequency of delivery, driver salesmen's wages, commissions, cabinet investments, size of cabinets, and size of new equipment. Thirty-two per cent of the replies from manufacturers indicated 3 deliveries per week in summer and 2 in winter was most efficient. Various other combinations, up to daily delivery, were suggested. Location of store with respect to the plant or on a route was listed most often as influencing the number of deliveries which should be made. There was no uniformity in replies regarding the frequency of delivery to accounts of different size. However, 47% thought that 5 gallons was the least amount that could be delivered economically and a majority thought that 100 gallons per year was the least amount of sales that justified an investment in a cabinet. In reply to the question regarding the preference for larger cabinets and trucks, 68% of the manufacturers stated that they would buy larger ones. Forty-five per cent of the manufacturers pay drivers a salary and commission. Commissions range from 1 to 6 cents per gallon.

W.H.M.

217. A study of ice cream delivery practices. ANONYMOUS. Ice Cream Trade J., 44, 1: 38-40, 73-74. Jan., 1948.

The high points of a survey of 1945 delivery practices of 350 companies made by the Statistical and Accounting Department of the International

Association are as follows: (a) The cost of distribution constituted a smaller share of the operation in 1945 as compared with 1941, dropping from 29.05% of the expense dollar in 1941 to 20.19% in 1945. Distribution cost includes delivery and customer service and selling. (b) Cost of products used in ice cream increased from 45.43% of the expense dollar in 1941 to 54.6% in 1945—an increase of more than 20% in cost, which was offset to a large extent by the drop in distribution cost. (c) Manufacturing and administrative costs in the 2 years were about the same. Administrative cost in 1941 was 5.57% of the expense dollar and 5.76% in 1945; manufacturing costs were 19.95% and 19.37%, respectively. (d) More than four-fifths of the ice cream volume (87.09%) is handled via the peddle system in delivering ice cream. (e) Most cabinets from which ice cream is sold are loaned to dealers with no rental charge; 61% of the cabinets were in this category. However, a surprising number of cabinets (18.6%) were rented to dealers and 20.4 were owned by the dealers. (f) The manufacturers who use 2 of the foregoing methods of cabinet service sell 53.88% of the ice cream; 16.79% use all 3 methods; 20.19% use only 1 method; and 9.14% did not answer. (g) The frequency of delivery was limited by 87.12% of the ice cream manufacturers, and this group sold 90.99% of the ice cream. (h) Of those answering the survey, about 47.5% felt that 5 gallons was the least amount that could be delivered economically; 26.02% thought 10 gallons; 6.85%, 15 gallons; and 4.11%, 20 gallons. However, 5.93% felt that they could deliver as little as 2.5 gallons economically. (i) Some 45.71% of the ice cream manufacturers pay a salary and commission to their driver salesmen; 5.4% pay a commission only. The most popular commission was 1 cent per gallon, with 2 cents per gallon the next most popular method.

W.H.M.

- 218. "Dry stop" merchandising equipment makes its debut.** ANONYMOUS. *Ice Cream Trade J.*, 44, 1: 46-47, 70-73. Jan., 1948.

"Dry stop" merchandising equipment, with its display and advertising facilities, not only enables the "dry stop" dealer to direct attention to his ice cream products, but also gives him an opportunity to promote the sale of factory-filled packages on a greater scale than ever before and provides him with the means for selling cones, sundaes, frappes, hot fudge, carry-out bulk and other items not requiring carbonation. A detailed description of suitable units is given.

W.H.M.

- 219. Package production for consumer acceptance.** J. C. PFEFFER, G. P. Gundlack & Co., Cincinnati, Ohio. *Ice Cream Trade J.*, 44, 2: 52, 54, 83-85. Feb., 1948.

Sanitation, package design, and mix composition are among the factors that will influence favorably the sale of packaged ice cream. There seems

to be a demand for both the regular 12% and the deluxe high-fat, low-over-run ice cream.

The standard package is one of about 12% fat and 39% total solids, with about 75 to 80% overrun and frozen in a continuous freezer and hardened quickly in 6 to 7 hr. The purpose of the deluxe package is to make a product which will be accepted by the consumer who has demanded bulk dipped ice cream.

W.H.M.

220. 1947 gallonage. ANONYMOUS. *Ice Cream Trade J.*, 44, 2: 48, 95, 96. Feb., 1948.

The 1947 production of ice cream was 622,400,000 compared to 708,913,000 in 1946, or a 12% drop, according to the preliminary report of the U.S.D.A. Bureau of Agricultural Economics.

W.H.M.

221. Price trends. V. M. RABUFFO. *Ice Cream Trade J.*, 44, 1: 34-35, 64-68. Jan., 1948.

The sky-rocketing cost of ice cream ingredients, together with other increases, has resulted in an increase of 8 to 15 cents per gallon for bulk and packaged ice cream in most of the larger cities. Typical selling prices for bulk vanilla ice cream are as follows: New York City, \$1.66; Up-state New York, \$1.60 to \$1.65; Boston, \$1.60 to \$1.65; Chicago, \$1.76; Los Angeles, \$1.45; Milwaukee, \$1.55; Minneapolis, \$1.15; South, \$1.50; Philadelphia, \$1.52; Pittsburgh, \$1.47; and Cincinnati, \$1.45. Pint packages are priced in New York at \$1.86—up 16 cents per gallon, and the higher-fat French type was up 24 cents per gallon, to \$2.24. Retail prices generally have advanced from 2 to 5 cents on factory-filled pints.

W.H.M.

MILK

222. Off-flavors in market milk. A. A. SCHOCK AND D. F. BREAZEAL, S. Dak. State Coll. *Milk Plant Monthly*, 36, 9: 28-30, 32, 48-49. 1947.

The authors summarize the causes of off-flavors of milk. The milk off-flavors are classified as follows: (a) those transmitted by the cow, (b) those absorbed directly by the milk from the atmosphere, (c) those that gain entrance into milk directly from bacterial contamination, and (d) those resulting from enzymatic, chemical and photochemical changes occurring in the milk. In order to provide consumers with wholesome milk having a pleasing flavor, milk processors should pay especial attention to intake selection of milk, cleanliness of equipment, stainless steel construction, processing temperatures, and a daily check on the bottled product. Special emphasis is placed on the importance of having a well trained man who knows milk flavors at the receiving platform.

G.M.T.

- 223. Determination of time taken in three phases in a H.T.S.T. pasteurizer.** J. V. PASCOE. *Australian J. Dairy Technol.*, 3, 1: 3-5. 1948.

A. 0.1% water solution of methylene blue was introduced into the pipe from milk pump to regenerator by dissembling when the machine, running on water, was stopped. Upon resumption of pumping, 3-oz. samples were taken at 3-second intervals entering the holder from the heating plates, entering the regenerator after holding and leaving the regenerator before entering the brine cooler. Dye concentrations in the samples were determined with a Zeiss Pulfrich Photometer, after necessary dilution. The average holding time was 37.5 seconds, but some material came through in 18 seconds.

F.E.N.

- 224. A laboratory high-temperature short-time pasteurizer.** J. V. PASCOE. *Australian J. Dairy Technol.*, 3, 1: 5-7. 1948.

An apparatus based upon rapid and controlled changes in temperature of the heating and cooling water surrounding aluminum tubes containing 1.5 ml. of milk agitated by aluminum plungers within the tubes is described. The apparatus has been used to duplicate heating, holding and cooling times of commercial pasteurization equipment.

F.E.N.

- 225. Cream bodying.** G. G. GIBSON, Sidney Wanzer and Sons, Inc., Chicago, Ill. *Milk Dealer*, 37, 5: 43, 44, 100. Feb., 1948.

Methods by which the body or viscosity of cream can be increased are reviewed and discussed. A practical method based on 12 yr. of experience is outlined. Separate the cream as near the desired test as possible but always be sure that the fat is high enough for the desired test. It is much easier and better on the cream body to bring the test down by adding milk or skim than it is to use heavy cream to bring the test up. Cool immediately to 50° F. or less. Standardize and pasteurize. Cool to 40° F. or lower. Allow to stand for at least 0.5 hr., preferably 2 hr. or longer, if possible. Warm cream to 82-87° F., depending upon such things as season of year, type of vat, speed of agitation, and cream used. Hold 5 min., without agitation, after reaching desired temperature. Cool cream to 42° F. and watch cream closely from this point on in cooling; as soon as an increase in viscosity appears, stop agitation. If no increase occurs, cool cream to 38° F. Allow cream to stand at cooled temperature for at least 0.5 hr. or longer (up to 2 hr.) if possible. Just before bottling, agitate cream until desired viscosity is reached. This may take only 10 sec. or it may take 10 min., but at this point the cream can be put near the same viscosity every day. Check viscosity of the cream after it is bottled and again after 24 hr. to determine what kind of viscosity cream has when the consumer receives it.

C.J.B.

- 226. Ten common causes of excessive sediment in milk. Part 8. Milking machine suction cups.** C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. *Milk Plant Monthly*, 36, 9: 70-72. 1947.

The use of a milking machine does not necessarily insure freedom of extraneous matter in the milk, as indicated by farm sediment checks. Sediment found in milk indicates 3 principal sources of contamination, namely, dirt on the teats; suction cups dropping down during the milking process, where they pick up shavings, bedding and other extraneous matter; and from careless storing of the suction cups themselves. G.M.T.

- 227. Three-day milk delivery.** ANONYMOUS. *Milk Dealer*, 37, 6: 40, 102-104. March, 1948

Three-day-a-week milk delivery in Columbus, Ohio, is no longer an experiment. Thoroughly tested on a marketwide basis since it was first introduced in Oct., 1943, 3-day delivery is enthusiastically endorsed by Columbus milk dealers, routemen, plant employees, and consumers. Milk is received from the farm 7 days a week. Sunday milk-receiving requires a skeleton plant crew on that day—enough men to operate the receiving room and storage department. All other plant men and all drivers have Sundays off.

The production advantages and disadvantages and delivery advantages and disadvantages are given. The problem of inadequate storage space in refrigerators of consumers and in plant storage rooms was partially solved through the adoption of the square bottle. C.J.B.

- 228. Billing retail customers.** E. THOM. *Milk Dealer*, 37, 5: 40-42, 132-136. Feb., 1948.

Sending out monthly statements to retail milk route customers can and does range from a highly complex accounting and bookkeeping system, complete in every detail, to a method over-simplified to a point where it consists merely of the routeman leaving a note stating "You owe \$9.20". A general picture of some of the forms now used in the industry and some of the advantages and disadvantages encountered by the users of these forms is presented. The information is based on a questionnaire sent out to a selected list of milk dealers, both large and small, in all parts of the country.

C.J.B.

SANITATION AND CLEANSING

- 229. A review of the literature pertaining to the chemistry of can washing.** L.L. LITTLE, E. F. Drew & Co., Inc., Boonton, N. J. *Milk Plant Monthly*, 36, 11: 22-24, 26, 38, 40-41. 1947.

Washing compounds for cans should have properties of detergency, in-

hibition of film and scale formation, and prevention of corrosion. Film formation can be prevented through the use of sequestering agents. Corrosion rate of metals increases rapidly with decreasing pH, there being no justification for the general belief that organic acids are inherently less corrosive than inorganic acids when compared on a uniform pH basis. The detergent properties of the various alkalis have been firmly established through many years of application in cleaning operations. Detergents comprising alkalis, condensed phosphates, and synthetic detergents may be expected to act on the soil through physical action, base exchange, electrochemical action, saponification, and surface activity. Acid cleaners may be expected to act on soils through dissolving milkstone, physical action, and surface activity. Forty-six references are cited. G.M.T.

230. **Planned dairy plant sanitation.** M. P. BAKER, Dept. of Dairy Industry, Iowa State College, Ames. *Milk Dealer*, 37, 6: 118-122. March, 1948.

The sanitation of dairy plants is discussed under inspection of equipment, operations, store rooms and other non-processing rooms, and premises. The article is summarized as follows: Plant sanitation is related to efficiency of operation, quality of products and to public relations. In a planned program of plant sanitation it is important to outline first the objectives, the procedures to be followed and the time of the activities connected with the program. The management should be actively interested and, whenever possible, take an active part. Records should be kept in detail; they help in measuring progress and also in organizing desirable changes as their need becomes apparent. C.J.B.

231. **Once upon a time there was a neglected milking machine.** J. KEENAN, Pennsylvania Salt Manufacturing Co. *Milk Dealer*, 37, 6: 42, 43, 94. March, 1948.

Good milk utensil care is summarized. Before milking, rinse all utensils with water containing 200 p.p.m. chlorine. Use same strength, hot, for cleaning cows' udders. Immediately after milking, rinse all utensils with clean cold water. Then brush all utensils with hot water containing a good soapless dairy washing powder, and suck it through the milker units. Hang cup units of milking machine on rack and fill with fresh lye solution. Use 4 level teaspoons of lye to each gallon of water. Drain after 20 min. in freezing weather. Dismantle milker at least once a week, clean thoroughly, and replace worn or faulty rubber. Twice a month, give rubber the hot lye soak treatment, using 1 heaping tablespoon of lye to each gallon of water. Scrub all parts after rinsing with a good milk stone remover solution. C.J.B.

MISCELLANEOUS

- 232. Excessive "oiling off" of frozen cream can be prevented.** G. M. TROUT, Michigan State College. *Food Freezing*, 2, 9: 628, 629, 641, 648. Aug., 1947.

The results of research of a number of workers, including that of the author, on the control of oiling-off of frozen cream are presented. Addition of 10 to 15% sugar prior to freezing, while the best practical control procedure, places limitations on the use of the cream. Quick freezing, rapid defrosting, separation of milk when the fat is in the solid state, and immediate freezing without holding the precooled cream, slightly improve the stability of the fat emulsion. Homogenization, effective in re-emulsifying oiled-off cream, does not stabilize the fat emulsion when such cream is frozen and thawed. L.M.D.

- 233. A quality control program for dairy plants.** J. H. HEALTH, Southern Dairies, Winston-Salem, N. C. *Milk Plant Monthly*, 37, 2: 57-59, 69. 1948.

A quality control program for dairy plants should include field, plant, and laboratory control. Field control should work toward the production of more high quality milk, convincing the producer that the plant is sincerely encouraging him to produce economically high quality milk. Plant control might well be summarized as good housekeeping. Laboratory control involves certain qualitative checks on quality of the milk, tests for butterfat, bacteria, phosphatase, acidity, coliform, bottle sterility, and homogenization efficiency. G.M.T.

- 234. How employer-employee relationships reflect good management.** J. W. POST, Armour and Co., Chicago, Ill. *Milk Dealer*, 37, 6: 76-82. March, 1948.

Nothing in business that affects profit and loss has changed so radically in the past 7 or 8 yr. as have the people who perform the details of the operation. The following favorable assets of a good employee are given and briefly discussed: Careful workmanship, experienced and interested in present work, industrious, rapid worker, exercises initiative and resourcefulness, versatile, cooperative with supervisors, cooperative with associates, economical, good housekeeper, makes friends, health, punctual, and practices safety rules.

The following functions of management are given: (a) To analyze, to determine what is to be done, how it is to be done, and when it is to be done. (b) To train representatives to perform properly the what, how and when of the operation. (c) To direct those operations as changes in outside influences (such as competition, markets, legislation and emergencies) require. The Taft-Hartley Act and employer development also are discussed.

C.J.B.

- 235. Water treatment in the dairying industry.** A. K. BEENIE. Australian J. Dairy Technol., 3, 1: 24-32. 1948.

Water softening by various methods, demineralization, clarification, cooling systems in which water is used, boiler water treatment, water for washing purposes and bacteriological treatments are discussed in relatively general terms. F.E.N.

- 236. The preparation of peaches for freezing. Part IV.** J. G. WOODROOF, ETHYL SHELOR, S. R. CECIL, AND IDA ATKINSON. Food Freezing, 2, 9: 632-634. Aug., 1947.

This excerpt from the Georgia Experiment Station Bulletin 251 gives details of prevention of browning of peaches. Ascorbic acid and citric acid are used in combination, and 4 formulas, 2 for home use and 2 for commercial freezers, are given. These formulas are recommended as the result of 2 yr. of experimentation with several dozen packs of several varieties of peaches. High conversion corn sirup was found to be fully as effective as 50% cane sirup for sweetening peaches, its use being limited to 50% of the total sugar solids in a pack. A brief discussion of packaging is given, bringing out the needs of containers for the various consumer usages. L.M.D.

- 237. Brine freezing strawberries in tin.** C. F. ELLIS AND J. B. WEGENER, Food Processing Research Station, TVA. Food Freezing, 3, 1: 22, 56. Dec., 1947.

A report is made of immersion freezing of 1:4 pack sliced strawberries in no. 10 cans in a 34% chromated calcium chloride brine, circulated at approximately -27°F . At the end of 60 min., the unfrozen core was 3 in. in diameter, 4.5 in. deep, and had a frozen circumference 1.5 in. in depth. The temperature of the unfrozen core had dropped to 42 from the initial temperature of 73°F . There was no definite freezing point, but solidification apparently was completed within the range of 22 to 24°F .

On the basis of these findings, a commercial freezer put into operation a can immersion freezer of 5,850 lb. per hr. capacity using 0 to -10°F . brine. Following 54 min. of immersion freezing, the cans of berries were washed free of brine in a water spray, put in cartons, and placed in a zero storage room which had adequate coil surface for the completion of freezing. Samples taken from time to time showed that at the end of 54 min. the unfrozen core temperature was 44°F . Approximately 60% of the heat removal necessary for freezing had been done by the time the cans were removed from the freezer and the temperature of the unfrozen contents had been lowered sufficiently to arrest fermentation. This method of operation had several commendable features: (a) a brine temperature of -10 to 0°F . allowed moderate suction pressures at the compressors; (b) after the first batch, there was continuous operation; (c) the major portion of heat ex-

traction load was handled in the freezer; (d) the capacity of storage rooms greatly increased without the danger of an excessive temperature rise; and (e) faster freezing resulted in rapid temperature drop through fermentation range compared to doing all freezing in the storage room even if designed for a heavy load. L.M.D.

238. **The truck-trailer industry moves to keep abreast frozen food progress.** J. B. HULSE, Truck-Trailer Mfrs. Assn., Washington, D. C. Food Freezing, 3, 1: 36-37, 56. Nov., 1947.

This is the first half of an article clarifying misconceptions existing as to the facilities available for truck transportation of frozen foods and setting forth progress that has been made in the design of low temperature truck-trailer bodies and their refrigeration to handle frozen food transfer safely and efficiently. The author emphasizes that the refrigerated trailer vehicle is not intended to be used as a food processing plant. The advantages and disadvantages of the existing refrigerating systems are given. L.M.D.

239. **Food Freezing's survey of state locker laws and regulations.** ANONYMOUS. Food Freezing, 2, 10: 693-695, 710, 712. Sept., 1947.

The rapid assumption of status as a public service agent is evidenced in the wide scope of state locker plant laws and regulations enacted since the adoption of the first such statute by Iowa in 1939. Today 22 states have specific locker laws, and all but a handful of the others provide control through adaptations of existing statutes to cover regulatory action by state departments. The main portion of the article embodies a tabular condensation of locker plant laws for the 48 states, showing a striking lack of uniformity and inadequacy of protection of the patron against poor practices. On the other hand, there is evidence of a growing tendency toward more implicit adherence to the technological advances in the food-freezing field. L.M.D.

240. **Steps in the handling of frozen fish in the freezer warehouse.** J. M. LEMON, Technological Sec., Division of Commercial Fisheries, Fish and Wildlife Service. Food Freezing, 2, 9: 606-608, 642-645. Aug., 1947.

A trial was made of flavor absorption of butter stored at 10° F. with packaged fish. The butter, ordinarily wrapped and 93 score, was placed in sealed tins also containing various species of fish fillets individually wrapped in cellulose-base moisture-vapor-proof material. One control sample was held by itself. In 337 days the control without fish scored 91, and 2 samples with sole and oysters scored 91.5, while 1 sample with salmon scored 89. Proper protection should prevent any odor absorption by butter. L.M.D.

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

241. **Biological properties and mouse virulence of *Streptococcus agalactiae* and Lancefield's group "B" streptococci from human sources.** A. POMALES-LEBRÓN, P. MORALES-OTERO, AND J. BARALT, School of Tropical Medicine, San Juan, P. R. Proc. Soc. Exptl. Biol. Med., 64, 4: 410-412. April, 1947.

The biological properties and mouse virulence of 70 Lancefield's group B cultures (50 bovine and 20 human) were studied and compared. Aside from the inability to ferment lactose by 8 of the human strains and some difference in the fermentation of glycerol, dextrin, and salicin, the fermentation reactions were very similar for both groups. With the majority of strains the differences between single bovine and human cultures were not greater than those between individual strains from the same source. There was an indication that human strains are more likely to reduce methylene blue than are bovine strains. Strains from human sources possessed a higher mouse virulence than those from bovine sources. R.P.R.

BREEDING

242. **Relation between time of fertilization and follicle cell dispersal in rat ova.** S. L. LEONARD, P. L. PERLMAN, AND R. KURZROK, Cornell Univ. Proc. Soc. Exptl. Biol. Med. 66, 3: 517-518. Dec., 1947.

Female rats were bred and 12-26 hr. later the ova were removed, examined for the disposition of the follicle cells, and then transferred to a microscope slide to determine if fertilization had occurred. Sixty-five ova, obtained 12-16 hr. post-coitus, were observed to be covered with follicle cells and remained in a compact mass when removed from the oviduct. The ova were similar in appearance to ova recovered from 100 non-bred rats. Spermatozoa were identified within the perivitelline space and polar bodies were present in every instance. Thirty-one fertilized ova, obtained from 10 rats (in 2 instances 13 hr. and in 8 instances 16-26 hr. post-coitus), either were partially or completely denuded of their follicle cells. In not one case were the ova denuded in 12 rats in which hyaluronidase was introduced into both uterine horns during estrus and the ova removed 18-24 hr. later. In all cases, the *in vitro* addition of the uterine fluid of these rats induced complete denudation in 10-20 min. It was concluded that fertilization in the rat ovum occurs before mass displacement of the surrounding follicle cells. R.P.R.

243. Effects of testis hyaluronidase and seminal fluids on the fertilizing capacity of rabbit spermatozoa. M. C. CHANG, Shrewsbury and Tufts Medical College, Boston. Proc. Soc. Exptl. Biol. Med., 66, 1: 51-54. Oct., 1947.

Sixty-five adult non-pregnant rabbits were superovulated by hormonal means and inseminated with a number of spermatozoa needed to fertilize only a small number of ova. Semen of a single male rabbit was collected with an artificial vagina and diluted 1:1,000 with saline. Sperm concentration was determined and saline or sperm added if the concentration of sperm was too high or too low. Then 0.5 ml. of such a suspension was added to each of the following: (a) 0.5 ml. of saline containing a known amount of hyaluronidase, (b) 0.5 ml. of supernatant fluid of normal semen after heating, (c) 0.5 ml. of semen from a vasectomized buck, or (d) 0.5 ml. of saline. One milliliter of these mixtures was inseminated intravaginally. The rabbits were killed 25-30 hr. later, ova flushed from the tubes, and the number of fertilized and unfertilized ova determined. The seminal fluid and not the hyaluronidase was found to have the ability to increase the fertilizing capacity of spermatozoa. R.P.R.

CHEESE

244. Sur l'accident du "Bleu" in fromagerie de pates molles a groute fleurie ("Blue" defect in cheese factories making soft, mold-cured cheese). J. KEILLING, J. CASALIS, JEANNE DUTHEN, L. SIGONNEY, AND IRENE GLASER. Lait, 27, 268: 461-466. Sept.-Oct., 1947.

Blue discolorations on the surfaces of soft cheeses of the Camembert, Coulommiers and Brie types frequently are a serious problem. *Penicillium candidum*, the surface growth commonly present, produces a white, felt-like coat, but growth of *Penicillium glaucum* will result in blue or green spots if sporulation occurs. Observations indicated that bluing did not take place uniformly on both surfaces but was more frequent on the surface which had received prolonged exposure to the anaerobic conditions during drainage. Analyses for ethyl alcohol indicated that this section of the cheese might contain twice to ten times as much as the section not showing the defect, the high alcohol content being the result of yeast growth. Control measures recommended include regular turnings during the draining period and stricter sanitation of draining tables. O.R.I.

245. Étude sur l'eau lié des fromages (Study of the bound water of cheeses). G. Mocquot. Lait, 27, 269-270: 576-595. Nov.-Dec., 1947.

The methods which MacDowall and Dolby developed for determining bound water in Cheddar cheese have been adopted for use on Gruyere curds.

The water-binding behavior of the calcium caseinate fraction, in particular, was studied. Finely cut curds were prepared by coagulating skim milk with rennet at 34° C.; the curds were cooked to 48–50° C. and washed in running water to remove all lactose and other solubles.

Bound water content was determined from a formula based on the extent to which various indicator solutions changed in concentration when the curds were soaked in them for 5 hr. at 10° C. Glycerol, C₆, C₁₂, and C₁₈, sugars, acetone and ethyl alcohol were used as indicator solutions, and bound water content was calculated on the basis of dry matter and on protein content. The values obtained with the sugars were related to their molecular weights, the di- and tri-saccharides yielding values approaching twice and three times as much, respectively, as those for glycerol and the mono-saccharides. Acetone gave values somewhat lower and ethyl alcohol gave values very much lower than the monosaccharides.

The influence of certain variations in cheesemaking methods also was studied. Increasing the degree of pressing and heating the curds decreased bound water content slightly. The addition of salt decreased bound water content greatly as compared to curds receiving no salt. O.R.I.

246. New dehydrated milk products for making soft types of cheese.

W. I. TRETSVEN, Chicago, Ill. *Milk Plant Monthly*, 37, 3: 38–42. 1948. Also, *Milk Dealer*, 37, 7: 44, 45, 106–112. April, 1948.

Cottage cheese production and demand largely is seasonal. This cheese may be stored in brine or in the frozen state for use when skim milk is not so abundant. Manufacture of cottage cheese from dehydrated milk products offers a possibility. Roller-dried nonfat skim milk powders are not suited because the reconstituted solids are quite insoluble and settle out, failing to coagulate during the cheesemaking operation. Spray-dried nonfat milk solids may be used in manufacturing cheese, but their use is somewhat limited because the coagulated reconstituted milk may not shrink adequately to yield a good quality cheese. Several variations in reconstituting the nonfat dry milk solids for cottage cheese making may be employed. New products are on the market which, when added to the reconstituted milk, aid materially in cottage cheese manufacture. The use of Lactal results in an increased yield of cheese and a reduction in losses in the whey; whey disposal problems are alleviated. While developed primarily for manufacturing cottage cheese, Lactal has other possible uses, such as, for carrying mother culture and starters; for making Baker's cheese, which drains readily without being sticky or pasty; for manufacturing Newfchatel and cream cheese; and for emulsifying dry milk fat into the reconstituted product for creaming cottage cheese. G.M.T.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

247. Dry milkfat as a form of storage fat in the dairy industry. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 43, 57. 1948.

Dry milkfat as opposed to butter oil is produced from fresh cream. The cream is heated to a temperature ranging from 170–190° F. and separated into 80% fat. By running the plastic cream through a homogenizer into a continuous type settling tank, a product containing approximately 98% fat and 2% moisture is secured. By centrifuging, a dehydrated milkfat is obtained. The standard of the U. S. Army Quartermaster Corps requires not less than 99.8% milkfat in the final product. Such milkfat may be stored at 40° F. for a period of at least 6 months with little analytical or organoleptic change. Dry milkfat may be used in any place that butter can be used. In areas lacking proper refrigeration and where there is a shortage of milk supply, a combination of nonfat dry milk solids and dry milkfat makes possible most types of dairy products. Dry milkfat should not be confused with butter oil.

G.M.T.

248. Sur la synérèse des caillés lactiques obtenus par l'action de *Streptococcus thermophilus* (On the syneresis of lactic curds as a result of the action of *Streptococcus thermophilus*). J. KEILLING, J. CASALIS, AND IRENE GLASER. *Lait*, 27, 268: 449–461. Sept.–Oct., 1947.

In the manufacture of yoghurt and similar products, the separation of whey is undesirable. A mixture of *Streptococcus thermophilus* and *Thermobacterium yoghurt* is used in making yoghurt. The authors have investigated the effects of such factors as rates of heating, pasteurization temperature, length of holding time, and rate of cooling upon the degree of whey separation in cultures of *S. thermophilus* incubated at 45° C. Whey separation was at a minimum where the milk was heated either rapidly or slowly to temperatures above 84° C. with no holding period. At 80° C. whey separation was negligible with a holding time of 10 min. or longer.

O.R.I.

DISEASES

249. Bovine brucellosis—A problem for the whole dairy industry. G. R. SPENCER, Dept. of Vet. Med., Univ. of Wis., Madison. *Milk Plant Monthly*, 37, 5: 42–46. 1948.

Brucellosis, formerly known as Bang's disease or contagious abortion, is responsible for sterility and lower production of milk, both of which are of great economic importance to the dairy industry. Pasteurization of milk

will kill *Brucella* organisms and is a positive protection to consumers. Nevertheless, precautions must be taken to protect animals from infection with the organisms. The bacteria causing the disease are taken into the body with food or water. Animals infected with *Brucella* may recover yet remain as carriers, spreading the infection throughout the herd. Nation-wide attention is given toward control. Three methods of brucellosis control are in use at the present time, namely: (a) test and slaughter, (b) use of quarantine, sanitation and restriction of the movement of infected cattle, and (c) vaccination with a live culture of *Brucella* called *Strain 19* to produce resistance to the disease. An agglutination test known as the "ring" or Fleisheauer test promises unusual possibility in detecting brucellosis-infected milk at the receiving platform. Research is underway, particularly by Dr. I. F. Huddleson, Michigan State College, to develop better vaccines than are available at the present time. The success in virtual elimination of bovine tuberculosis from the dairy cows offers an incentive to the possibility of eradication of brucellosis from the dairy herds. G.M.T.

FEEDS AND FEEDING

250. Essai d'augmentation de la matiere grasse du lait par l'administration de vitamines du groupe B contenues dans la levure de biere (Attempt to increase milk fat by the administration of the B group vitamins in brewer's yeast). D. GIANNOTTI, Pisa University. Lait, 27, 269-270: 561-576. Nov.-Dec., 1947.

Brewer's yeast at rates of 5, 10 and 20 g. per day was fed in a paired feeding and production test over a 2.5-month period. The results indicated that neither fat content of the milk nor milk production was augmented by these additions to the ration. O.R.I.

251. Feeding urea to dairy cows with special reference to the palatability of feed mixtures containing urea. J. E. BOWSTEAD AND H. T. FREDEEN, Univ. of Alberta, Edmonton. Sci. Agr., 28, 2: 66-78. Feb., 1948.

Results of practical feeding trials carried out over a number of years in the use of urea as a protein substitute in dairy cattle feeding are reported. Attempts to compare the value of urea at a level of 2% in a ration resulted in failure due to lack of palatability. Cows suddenly switched to a ration containing 2% urea and those gradually introduced to such a ration consumed their grain allowance without relish and after 12 weeks developed an aversion to rations containing urea. The inclusion of corn made the ration somewhat more palatable, and molasses increased palatability considerably. The authors suggest that this aversion is not due to taste but to the possibility that the bacterial flora of the rumen is altered when urea is fed.

Individual cows vary greatly in their tolerance for urea, although a grain ration containing 0.5% is eaten fairly readily. The addition of cobalt possibly may be an aid in the feeding of urea. O.R.I.

252. Digestibility studies with ruminants. XI. The effect of the nutritive ratio of a ration upon its digestibility by cattle. C. J. WATSON, J. W. KENNEDY, W. M. DAVIDSON, C. H. ROBINSON, AND G. W. MUIR, Central Experimental Farm, Ottawa, Canada. *Sci. Agr.*, 27, 12: 600-608. Dec., 1947.

Using timothy hay of good quality, barley, and soybean oil meal, mixed rations were made up with nutritive ratios varying from 1:2.63-1:8.41. Eight rations were used in the experiment. The coefficients of digestibility of these rations were determined with 8 grade Shorthorn steers. The coefficients of digestibility of the individual feeds also were determined, and these were used to calculate the coefficients of digestibility of the mixed rations. By comparing the coefficients so calculated with those obtained in the digestion trials, it was shown that the nutritive ratios of the mixed rations did not influence their digestibility. O.R.I.

253. Epithelial keratinization as evidence of fetal vitamin A deficiency. J. G. WILSON AND J. WARKANY, Univ. of Rochester and Univ. of Cincinnati. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 419-422. April, 1947.

Keratinizing metaplasia was found in epithelia of the genito-urinary tract of fetal rats taken from mothers that were deficient in vitamin A during pregnancy. Prior to the 18th day of gestation, stratified, keratinized epithelium was not found; however, all fetuses older than 18 days showed keratinizing metaplasia in at least some of the genito-urinary epithelia. By the 20th day keratinization was seen in the dorsal wall of all organs distal to the termination of the genital ducts. Epithelia in other parts of the body were not affected. R.P.R.

254. The stability of iodine in iodized rock salt. W. M. DAVIDSON AND C. J. WATSON, Central Experimental Farm, Ottawa, Canada. *Sci. Agr.*, 28, 1: 1-5. Jan., 1948.

Blocks of sodium chloride containing added potassium iodide at the rate of 1 oz. per 5 lb. of salt were put up by a commercial manufacturer. Protective agents such as calcium stearate and sodium thiosulfate were added to some mixtures and ferric oxide was added to others. Granulated salt representative of each mixture also was stored in glass jars.

Blocks of each of these mixtures were exposed to summer pasture conditions for 2 months, to conditions simulating stall feeding for 16 months, and to storage in cartons at barn temperatures for 9 months. The granulated salt was examined after 3 and 13 months.

Under summer pasture conditions complete disappearance of the iodine took place in 2 months from the blocks, even when protective agents were present. Under stall conditions the iodine showed fair stability for 2 months, but only small amounts remained after 17 months. In cardboard cartons there was no loss of iodine over a 9-month period, and iodine in the granulated salt remained stable for 15 months. O.R.I.

- 255. Method of controlling the growth and fattening rate of livestock and poultry and composition used in connection therewith.** C. W. TURNER AND E. P. REINEKE. (Assigned to American Dairies, Inc., and Quaker Oats Co.) U. S. Patent 2,438,353, March 23, 1948 (13 claims). Official Gaz. U. S. Pat. Office, 608, 4: 769. 1948.

Livestock and poultry may be grown and fattened at an increased rate, without affecting milk or egg production, by feeding during a selected span of life, thyroprotein (example: iodinated casein) which stimulates metabolism and then feeding substances containing the thioureyline radical (example: thiouracil) in the range of 0.01 to 0.15% for each 100 lb. of feed to arrest thyroid activity. R.W.

FOOD VALUE OF DAIRY PRODUCTS

- 256. The nutritive value of commercial ice cream.** A. C. DAILBERG AND J. K. LOOSLI, Dept. of Dairy Industry and Dept. of Animal Husbandry, Cornell Univ., Ithaca, N. Y. Ice Cream Trade J., 44, 4: 56, 99-101. April, 1948.

Three batches of commercial ice cream were made, the composition being as follows: fat, 12%; nonfat milk solids, 10%; sugars, 15.5%; gelatin, 0.3%; egg solids, 0.2%; and total food solids, 38%. The ice cream was found to contain the following nutrients per 100 g. of ice cream: calories, 206; protein, 3.85 g.; fat, 12.06 g.; carbohydrate, 21.31 g.; total minerals, 0.81 g.; calcium, 0.112 g.; phosphorus, 0.105 g.; iron, 0.120 mg.; thiamine, 0.038 mg.; riboflavin, 0.236 mg.; niacin, 0.098 mg.; vitamin A, 548 I.U.; and ascorbic acid, 0 mg. W.H.M.

- 257. Calcium enriched meat compared with milk as source of calcium, phosphorus and protein.** I. MCQUARRIE, MILDRED ZIEGLER, AND I. H. MOORE, Univ. of Minn. Medical School. Proc. Soc. Exptl. Biol. Med., 65, 1: 120-121. May, 1947.

From the viewpoint of Ca, P and protein, the nutritional value of a Ca-enriched meat diet was compared experimentally with a milk diet containing the same quantities of essential food constituents. Balance studies were carried out in 2 young male patients. Total carcass and separate femur

analyses were made on rats which were on 2 types of diet. The results indicated that the Ca-enriched meat diet was equally as good as the milk diet as a source of Ca, P and protein. R.P.R.

- 258. Dental caries in the cotton rat. IX. Effect of milk rations. E. P. ANDERSON, J. K. SMITH, C. A. ELVEHJEM, AND P. H. PHILLIPS, Univ. of Wisconsin. Proc. Soc. Exptl. Biol. Med., 66, 1: 67-69. Oct., 1947.**

Cotton rats were placed on experiment at weaning (20-25 g.). Rats from each litter were distributed as equally as possible between the control and experimental groups. Each experiment lasted for 14 weeks, during which time body weights were recorded at biweekly intervals. At the end of the experiment the animals were killed and the incidence and extent of carious lesions estimated. Milk was found to be protective against dental caries, and zero scores in rats fed only liquid milk were obtained. Animals that received milk to which sucrose, glucose, or dextri-maltose (sugars that produce caries when fed in dry rations) had been added exhibited low caries scores as compared with controls on a cariogenic ration. The indices of dental caries in rats receiving approximately one-third of their caloric intake as liquid milk and the remainder as a cariogenic ration were less by 50% than those of litter-mate controls not receiving milk. R.P.R.

- 259. Fractionation of lacteal liquids. F. K. DANIEL. (Assigned to Sun Chemical Co.) U. S. Patent 2,437,080, March 2, 1948 (5 claims). Official Gaz. U. S. Pat. Office, 608, 1: 154. 1948.**

Cold milk, skim milk, whey, etc., may be continuously fractionated into 2 fractions by an osmotic diffusion and stratification dialysis procedure, employing a series of cells separated by cellophane or other semi-permeable membrane. The proteinaceous fraction, consisting of undenatured casein, lactalbumin, and lactoglobulin, is suitable for the diet of those suffering from pancreatin deficiency or diabetes, for use in ice cream, as a whipping aid, emulsifying agent, etc. The diffusate, consisting of the lactose, ash, water and soluble vitamins, is suitable as a culture medium for micro-organisms and as a source of vitamins and other compounds. R.W.

ICE CREAM

- 260. Selling through franchise dealers. E. THOM. Ice Cream Rev., 31, 9: 40, 41, 88, 90. April, 1948.**

The program of the Bridgeman Creameries for building sales of ice cream through a selected group of 22 franchise dealers in Minneapolis and St. Paul is discussed. Franchise dealers are required to sign a contract

which will insure full compliance with Bridgeman's merchandising program. The equipment to be installed and its arrangement, the training of fountain personnel, advertising and promotional activities, the sanitary standards to be maintained, and the items to be sold or featured all are controlled by the company. The dealer, in turn, is assured a satisfactory profit on his fountain operation. Factors which tend to attract trade to the stores are: Customers receive full value for their money; a rule at all Bridgeman stores is generous servings reasonably priced. The quality of ice cream is as high as any in the market and the product is maintained under ideal storage conditions. The store surroundings are conducive to the greater consumption of ice cream. Customers are reminded of ice cream whenever they walk into a Bridgeman store. Ice cream is the featured item at all stores. The dealer actively promotes the sale of ice cream.

W.J.C.

261. The retail store, past—present—future. Part 3. D. GHORMLEY.
Ice Cream Trade J., 44, 4: 48-49, 88-92. April, 1948.

Findings of the Stanford University survey show a marked increase in the number of retail ice cream stores since 1946. The average gross sales per store in 1946 were \$5,250 per month compared to \$4,190 per month in 1940. The annual sales volume per store reporting was 24,225 gallons.

Forty-one per cent of the sales of such stores are to minors, 45% to persons between 21 and 50 yr. of age, and 14% to persons over 50 yr. of age. Seventy-four per cent of purchases are made by women. Fifty per cent of specialized retail ice cream stores are located in suburban business districts. Fifty-eight per cent of customers drive to stores, indicating the value of parking lot facilities. Ninety per cent of store operators favor parking lots on the premises. Fifty-five per cent of the firms reporting use training manuals for help. Employees are 64% women, 20% men and 16% minors. Sideline products have come to be accepted in virtually all stores. W.H.M.

262. Consumer buying habits on ice cream. ANONYMOUS. Ice Cream Trade J., 44, 4: 44-45, 103, 104. April, 1948.

The Chicago Tribune has made a survey by telephone of 271 families in Chicago to determine when, where and by whom ice cream purchases are made. More than 50% of the purchases were made on Friday and Saturday. In 53.1% of the cases the ice cream purchased was served at a meal, usually at the evening meal. The heaviest buying period was from 3 to 9 p.m., with the peak at 4 to 7 p.m. The housewife made 45.1% of the purchases, the husband 19.9% and the children made the remaining purchases. When the husband made the purchases, in two-thirds of the cases he did so after he returned home from work. The two chief types of stores where pur-

chases were made were the drug store in 31.4% and the grocery store in 27.7% of the cases. Convenience was given as the main reason for making the purchase at a particular type of store, followed by brand preference in about one-third of the cases.

A large portion of the ice cream going into the home still is not a part of the daily menu but rather is being used as a mid-afternoon snack or as a refreshment late in the evening. With convenience cited as the main reason for the purchase at a particular outlet, the grocery store in time may be the major outlet.

W.H.M.

263. **Sensible pricing at the ice cream soda fountain.** ANONYMOUS. *Ice Cream Rev.*, 31, 9: 44-45. April, 1948.

Reduction in ice cream gallonage in recent months is attributed in part to the practice of over-pricing soda fountain items by some dealers. It is not uncommon for the cost of ice cream to the consumer to advance well over \$1.00 per gallon at the soda fountain when the wholesale price of ice cream advances by only 15 cents per gallon. Such practice tends to price the soda fountain out of business. An educational program with dealers is suggested as the best method to combat this practice. Supplying dealers with concrete cost information, showing exact cost figures for individual servings of ice cream, and toppings at varying prices per gallon for ice cream and toppings have proved very successful in holding soda fountain price structures in line, according to the reports of one company. Two charts are reproduced in the article and should prove valuable to anyone interested in discussing fair price structures at soda fountains with their dealers. A 45% gross profit is considered ideal for soda fountain operation, with the desirable range from 40 to 50%.

W.J.C.

MILK

264. **Continuous flow pasteurizer.** H. I. SOUTHERWICK. (Assigned to the Do All Co.) U. S. Patent 2,438,582, March 30, 1948 (4 claims). *Official Gaz. U. S. Pat. Office*, 608, 5: 949. 1948.

In a simple sanitary continuous pasteurizer for such fluids as milk, the heating is accomplished by the resistance offered by the milk to an electric current as it flows between two electrodes. The electrodes are cooled by the incoming cold milk. Close temperature control is maintained by altering the rate of flow of the milk by means of a valve which is actuated by the magnitude of the current flowing through the liquid. This arrangement automatically compensates for any fluctuation in the current supply and changes in the resistance of the milk due to temperature.

R.W.

- 265. Production of recombined and reconstituted milk.** O. E. STAMBERG. Industrial Research Laboratories, Milwaukee, Wis. *Milk Dealer*, 37, 7: 47, 118-120. April, 1948.

The use of reconstituted whole milk, a product made by properly dispersing dry whole milk powder in the required amount of water (the term sometimes is employed for a product made from evaporated milk and water) and of recombined whole milk, a product made by properly combining non-fat dry milk solids with cream, butter or milk oil and the required water, is discussed. These products all are nutritionally excellent. Reconstituted milk produced from evaporated milk has a pronounced cooked flavor which detracts from its acceptance for drinking purposes. Whole milk powder must be used quite fresh; otherwise the milk will have off-flavors, referred to generally as "chalky", "oxidized", or "tallow". The production of recombined milk requires more technological knowledge than the reconstitution of whole milk powder, but it generally is conceded that recombined milk is superior in quality to most products from whole milk powder.

C.J.B.

- 266. Promoting greater milk production.** M. H. BRIGHTMAN. *Milk Dealer*, 37, 7: 42, 43, 98-100. April, 1948.

Charts are presented and discussed showing that prices of dairy products historically have kept ahead of the prices of farm products other than dairy; income from dairy cattle remains more consistent, and usually higher, than that from either food or feed grains; and milk production is not keeping up with the human population of the United States. The feed situation is more optimistic than it was a few months ago. The author concludes that the dairy business is a good business, that it has returned more money in the past and probably will return more in the future than most other types of farming, that cows that are capable of producing milk efficiently should be kept, that good heifer calves should be raised, and that we should feed efficiently to get maximum milk production.

C.J.B.

- 267. Milk bottle utility device.** J. STRANSKY. U. S. Patent 2,438,024, March 16, 1948 (5 claims). *Official Gaz. U. S. Pat. Office*, 608, 3: 600. 1948.

A device for attaching to the top of a standard milk bottle is described which provides the following features: (a) a tube inserted through the disc cap which allows pouring of the milk without removing the cap and exposing the product to the exterior lip of the bottle, and (b) a receptacle for attaching notations to the person delivering the milk or for holding money for payment of the milk.

R.W.

PHYSIOLOGY

- 268. Effects of gonadotrophic hormones on lactation.** G. M. C. MASSON, Univ. of Montreal. *Proc. Soc. Exptl. Biol. Med.*, 66, 3: 506-508. Dec., 1947.

Forty-one lactating albino rats weighing 250-300 g. were divided into 6 groups, 5 of the groups receiving a gonadotrophin of either pituitary or chorionic origin. Pregnant mare serum had a strong inhibitory effect on lactation; only 18% of the litters survived until the 16th day. The gonadotrophic principle of the urine of pregnant women produced a slight inhibition of lactation, while an anterior pituitary preparation was inactive. Histological studies of the ovary, vagina and uterus indicated that the administration of pregnant mare serum resulted in a high level of estrogen and progesterone.

R.P.R.

- 269. Effect of sex hormones on pituitary lactogen and crop glands of common pigeons.** J. MEITES AND C. W. TURNER, Dept. of Dairy Husb., Univ. of Mo., Columbia. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 465-468. April, 1947.

Groups of 16 to 20 mature pigeons of both sexes were injected subcutaneously daily for 10 days with either estrone, diethylstilbestrol, progesterone or testosterone propionate. The pigeons were killed on the day after the last injection, weighed and examined under light for evidence of visual proliferation. The pituitaries of each group were removed, weighed, and assayed for their lactogen content by injecting them intradermally over the crop glands of 10 common pigeons. The results were all negative, and it was concluded that the pigeon pituitary, unlike the mammalian, is refractory to the administration of gonadal hormones.

R.P.R.

- 270. Effect of thiouracil and estrogen on lactogenic hormone and weight pituitaries of rats.** J. MEITES AND C. W. TURNER, Dept. of Dairy Husb., Univ. of Mo., Columbia. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 488-492. April, 1947.

The feeding of a ration containing 0.1% thiouracil for 24 days to young female rats reduced the lactogen content of the pituitaries below that in normal rats. In rats fed thiouracil for 14 days, the subsequent administration of thiouracil and 100 I.U. of estrone daily for 10 days failed to maintain the normal level of the lactogenic hormone. However, there was an increase in the lactogen content of pituitaries of rats that received estrone and thiouracil daily for 21 days. Pituitary weight was increased by the administration of thiouracil as well as thiouracil plus estrogen. Thyroid hypertrophy was less in rats receiving thiouracil plus estrogen than it was in rats receiving thiouracil alone.

R.P.R.

SANITATION AND CLEANSING

- 271. Some comparisons of the disinfecting properties of hypochlorites and quaternary ammonium compounds.** L. SHERE, The Diversey Corp., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 66-69. 1948.

A considerable variation in the germicidal effect of different quaternary compounds is reported. Increasing water hardness has a great and variable effect in reducing the germicidal power of different quaternary compounds. Anionic wetting agents commonly used in cleaning compounds affect adversely the germicidal action of quaternary compounds. Milk solids adversely affect the germicidal action of quaternary compounds and hypochlorite. However, with milk solids present, hypochlorite kills at a lower concentration than any of the quaternaries tested. Lowering the water temperature below 68° F. reduces the disinfecting power of quaternary compounds. Precipitation formed in hard water by the water softening action of certain alkalis absorbs the quaternary compounds and removes them from solution; these precipitates do not absorb hypochlorites. Increasing water hardness, addition of anionic wetting agent, and reduction of water temperatures below 68° F. have no adverse effect on the disinfecting action of sodium hypochlorite.

G.M.T.

- 272. Good housekeeping in the dairy plant.** J. H. ERB, The Borden Co., Columbus, Ohio. *Milk Plant Monthly*, 37, 4: 70-71. 1948.

Good housekeeping, beginning with a building in good repair, results from cooperative effort between the manager and the plant organization. A special housekeeping committee which makes regular monthly inspections and written reports in each plant is most helpful. Forms aid in calling attention to items which should be judged and inspected. Special items in plant housekeeping and operation which may reflect good housekeeping are: fit of valves, broken glass on floor, accumulation on sills, lack of paint, lack of dairy sanitation, excess grease on equipment, equipment not polished, untidy laboratory tables, dirty window panes, cleanliness of halls, condition of locker room, employee uniforms, freedom from insects and rodents, orderliness of stock room, and neatness and appearance of platforms. Dairy plants have made great progress in good housekeeping but much improvement still is needed.

G.M.T.

- 273. How to get cans clean.** V. SCHWARZKOPF, Lathrop-Paulson Co., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 46-51. 1948.

Before the milk can can be cleaned, one must have an understanding as to what constitutes a clean can. Such cans must look, feel and smell clean, be dry and be practically too hot to handle when released from the can

washer. Keeping the machine clean, maintaining optimum temperature, maintaining proper strength solutions, and keeping the machine in good operating condition will influence good can washing. The author summarizes the solution to the problem of getting cans clean as follows: Make someone responsible for the proper operation and care of the can washer, and see that this responsibility is fully assumed. Keep machine clean, well groomed and lubricated. Use temperatures for all treatments which will provide maximum cleaning, maximum destruction of bacteria and maximum dryness without lime or scale deposit. Never reach or exceed the critical temperature of the cleaning compound being used. Maintain proper strength solution for washing and rinsing to provide maximum cleaning without formation of lime or scale. Use relatively clean water for washing all cans. Select the cleaning compound most suitable for the can washer being used. Do not alternately use alkaline and acid cleaners if the temperatures used reach or exceed the critical temperature of the alkaline cleaner.

G.M.T.

MISCELLANEOUS

274. **Statistical evaluation of growth curves.** O. L. DAVIES, Imperial Chemical Industries, Manchester, England. *Proc. Soc. Exptl. Biol. Med.*, 66, 3: 567-568. Dec., 1947.

The comparison of growth curves by the method proposed by Weil is an oversimplified method of analysis and not a valid one. The *t*-test is a valid method, provided the apportionment of the animals between groups has been carried out in a strictly random manner. Some improvement probably would result when the final weights are corrected for the variations in the initial weight by the covariance method discussed by Fisher. A more complete method is to fit regression lines, by procedures such as the method of least squares, to the growth curve of each animal, using, if required, a simple transformation to the time and weight scale in order to produce a simple curve.

R.P.R.

275. **Statistical evaluation of growth curves.** C. S. WEIL, Mellon Institute, Pittsburgh, Pa. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 468-470. April, 1947.

Weight data were expressed in the form of frequency distributions, each weighing being considered a point in the distribution. The method of chi square revealed similar probability levels of significance, as did the ratio of 3 times the standard error of the difference, to the difference between the mean weights. The calculation of the coefficient of skewness gave values that justified the use of normal statistics. The use of the chi square test on the distributions was deemed the method of preference for showing differences in time-weight data.

R.P.R.

- 276. Radioautographs in which the tissue is mounted directly on the photographic plate.** T. C. EVANS, Columbia Univ. Proc. Soc. Exptl. Biol. Med., 64, 3: 313-315. March, 1947.

Tissue sections containing radioactive material were mounted directly on photographic emulsion in the dark room. After suitable exposure, the plate was developed and the tissue stained with Harris' hematoxylin and aqueous eosin, then dehydrated, cleared, and mounted in permount or balsam. The preparation was studied microscopically as the autographic image was in place just below the tissue. R.P.R.

- 277. Traffic cops of the pipelines.** H. J. BARTLETT, Crane Co. Milk Dealer, 37, 7: 52-60. April, 1948.

Check valves are designed to permit flow in one direction only; they close automatically if flow reverses. There are only two basic types, *i.e.*, swing check valves and lift check valves. Disregarding exceptions, it can be assumed that the greatest field of usefulness for swing check valves is in water and other liquid service. Lift check valves definitely are considered to be superior to swing check valves for service on steam, air, gas, and vapors in general. Different designs of each type are described. C.J.B.

- 278. This business of advertising.** R. E. SHANNON, The Evening Journal, Washington, Iowa. Milk Dealer, 37, 7: 158-159. April, 1948.

Advertising is discussed under the headings of sell personality, local advertising methods, and advertising suggestions. Know your audience; understand that they are more interested in themselves and their needs than they are in you. Prepare your advertising carefully; plan it well in advance and humanize it by using names and local references. Do not use heavy descriptions, waste words and space in claims of superiority, or try to educate the public in the technical phases of your business. C.J.B.

JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

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Ames, Iowa

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ABSTRACTS OF LITERATURE

BOOK REVIEW

- 279. Dairy bacteriology.** B. W. HAMMER. 3rd edn. 593 pp. \$6.00. John Wiley and Sons, Inc. 1948.

This new edition of the standard American book in this field reflects the increased amount of available material by including 111 pages more than found in the preceding edition, and by including material from a large number of recent publications. The chapter on "Tests for the General Quality of Raw Milk" has been moved almost to the front of the book, the chapter on milk-born diseases has been made considerably more concise and a chapter on "Bacteriology of Dairy-Plant Water Supplies" has been added, to mention only a few of the major changes. An increased number of illustrations and a greater tendency to point out the practical applications of the various test and control procedures help to make the book of greater value to both student and processor. The book is well-indexed and is printed and bound very satisfactorily. This new edition should be in the hands of all who are concerned with the technical aspects of the handling of dairy products, and many others could profit very considerably by greater familiarity with the material presented.

F. E. Nelson

BACTERIOLOGY

- 280. A comparative study of commonly used staining procedures for the direct microscopic examination of milk.** B. S. LEVINE AND L. A. BLACK, U. S. Public Health Service, Cincinnati, Ohio. *J. Milk and Food Technol.*, 11, 3: 139-148. May-June, 1948.

An aqueous methylene blue dye is readily incorporated in the milk proteins and may cause frequent overstaining. Strong contrasts are attained at the expense of delicate color shades, resulting in the loss of visibility of bacteria whose affinity for the dyes is comparable to the milk proteins forming the background of the smear. Sulfuric acid causes a distortion of the cells. Hydrochloric acid appears to cause a denaturation of the milk proteins, resulting in loss of adhesive properties. All the acids studied, including acetic, cause a light background of the stained smear, thus making lightly stained bacteria imperceptible to the eye. Methylene blue and basic fuchsin solutions require a high acidification, resulting in many disadvantages, while the red background is fatiguing to the eye. On the basis of these studies the authors believe that procedures for staining milk smears can be improved.

H. H. Weiser

- 281. Common micro-organisms in defective milk products and their control.** R. V. HUSSONG AND W. O. NELSON. *Can. Dairy Ice Cream J.*, 26, 10: 28-30. Oct., 1947.

The article gives the methods of staining, the types of bacteria found in dairy products and the microscopic appearance of these micro-organisms.

H. Pyenson

- 282. Recent developments in milk quality control.** A. R. M. MACLEAN. *Can. Dairy Ice Cream J.*, 27, 5: 31. May, 1948.

The most reliable test for the pasteurizability of milk is that of laboratory pasteurization. A plate count of 50,000 colonies per cc. is regarded as plant pasteurizable. For practical control, the plate count should be regarded as an index rather than a standard for the quality of pasteurized products.

H. Pyenson

- 283. Microlysine-tear gas preserves milk.** N. E. GIBBONS AND HELEN J. BROWN. *Can. Dairy Ice Cream J.*, 27, 5: 36-37. May, 1948.

Microlysine is a pure form of trichloronitro-methane for use in food products. It is better known as chloropicrin, one of the tear gases. Microlysine was used during the war in France to preserve milk. At all concentrations used, the numbers of viable organisms were greatly reduced. The reduction was roughly proportional to the concentration of microlysine, and the organisms growing at 98° F. were affected more than those growing at 70° F. No information is available about its effect on pathogens. Its use in milk is prohibited in Canada.

H. Pyenson

- 284. Possible uses of ultraviolet radiation in the dairy industry.** E. I. MORWICK. *Can. Dairy Ice Cream J.*, 27, 3: 34-36. March, 1948.

A new and practical method of controlling mold, bacteria and other micro-organisms is through the use of selected ultraviolet radiation. In the dairy industry sterilamps can be used in the cow barn to reduce the number of bacteria in the air and thus reduce the number of bacteria in the milk. Other uses are for the milk room, milk cans, and in the distributors plants over the bottle filler, conveyors and in those cases where milk is exposed to the air.

H. Pyenson

BUTTER

- 285. Studies on the Fritz butter machine.** J. A. PEARCE. *Can. Dairy Ice Cream J.*, 27, 4: 48-49. April, 1948.

Factors with no effect on production are: (a) the use of pasteurized or unpasteurized cream; (b) the use of a summer and winter cream; (c) the

use of cream with various acidities; (d) decreasing the number of paddles in the churning chamber; (e) allowing the butter granules longer passage in the butter press; (f) 16 alterations in the kneading chamber; and (g) width of the openings in the variables gates. Factors affecting butterfat loss were anti-clockwise rotation of the paddles and low paddle speed. Factors affecting production were increased fat content, cream temperature, the position of the valve in the constant level tank, rotation of the paddles, speed of the paddle, and jacket temperature. Factors affecting moisture content are cream temperature, the position of the valve opening, rotation of the paddles, jacket temperature, the temperature of the butter granules, and the auger speed. The Fritz butter contained more air than the sample of churn butter.

H. Pyenson

- 286. Report on the preparation of butter samples.** H. J. MEURON, Food and Drug Administration, San Francisco, Calif. J. Assoc. Offic. Agr. Chemists, 31, 2: 318-327. 1948.

Collaborative results of a study of methods for the preparation of butter samples for analysis are presented. The following method was adopted as official: Soften the sample by warming to 39° C., shaking intermittently to reincorporate any separated fat. When optimum fluidity is attained, shake vigorously at frequent intervals until the sample cools to a homogeneous semi-liquid of the consistency of thick cream. Optimum fluidity is described as that point where the emulsion is still substantially intact but the mixture moves freely on shaking. Weigh portion for analysis promptly.

F. J. Babel

- 287. Fat losses in creamery operation.** C. G. OBEE. Can. Dairy Ice Cream J., 27, 2: 27-28. Feb., 1948.

Since the butter industry is working on a small margin of profit, the losses must be kept down to a minimum. It is important to prevent and minimize losses in composition; errors in weighing and testing cream; losses in cans and weigh cans; cream used for samples; improper rinsing and draining of vats, pumps, and pipes; spills from cans, vats and churns; and losses with some mechanical printers.

H. Pyenson

CHEESE

- 288. Pasteurization of milk for cheesemaking.** R. W. BROWN. Can. Dairy Ice Cream J., 26, 10: 26-27. Oct., 1947.

The quality of the cheese as indicated by the scores for flavor is definitely in favor of the pasteurized milk cheese. There were 888 flavor scorings with 344 comparisons made on 296 samples of cheese, half of

which were made from raw milk and half from pasteurized milk. On the basis of these comparisons, the cheese made from pasteurized milk showed an average betterment in the flavor score of 1.198 points, or a value that frequently meant the difference between first and second grade cheese.

H. Pyenson

289. **How to increase yield in cheesemaking.** E. C. DAMBROW. *Can. Dairy Ice Cream J.*, 27, 2: 50. Feb., 1948.

To increase yield in cheesemaking refuse off-flavor milk at the intake, cut curd slightly on the soft side, use a metal plate to shove off curd sticking on the sides of the vat and use mechanical agitating instead of rake stirring. Mechanical agitation will increase yield about 25 lb. of cheese per 10,000 lb. of milk over rake stirring.

H. Pyenson

290. **Correcting defects in Canadian cheese.** J. M. BAIN. *Can. Dairy Ice Cream J.*, 27, 5: 58. May, 1948.

The main defects encountered in Canadian cheese are fruity, not clean, openness, rancid and extraneous matter. To overcome these defects cheese factories need better light, ventilation, sanitary equipment and closed-off boiler rooms.

H. Pyenson

291. **Studies on openness in Cheddar cheese.** E. G. HOOD AND C. A. GIBSON. *Can. Dairy Ice Cream J.*, 27, 3: 31-33. March, 1948.

When 1 hr. or longer was allowed between milling and salting, 100% of the experimental cheese were graded as close. When less time was allowed between milling and salting, 89.5% of the cheese were faulted to some degree for openness.

H. Pyenson

292. **Improving starters to make uniform cheese.** E. C. DAMBROW. *Can. Dairy Ice Cream J.*, 26, 10: 58. Oct., 1947.

For a good starter and uniform cheese, the following simple rules should be followed: (a) clean and sterilize starter equipment thoroughly with clean water in a clean wash sink; (b) use this equipment, thermometers, pails and containers for starter handling only; (c) eliminate off-odors from floors or sewer odors in plant; (d) do not let your breath come in contact with the starter or mother starter; and (e) get a fresh starter weekly from a reliable source.

H. Pyenson

293. **Extraneous matter in Canadian Cheddar cheese.** R. THIBODEAU. *Can. Dairy Ice Cream J.*, 26, 10: 31-33. Oct., 1947.

A new modification of the citrate method of detecting extraneous matter in cheese has been developed using a smaller sample of the size pro-

duced by a trier and may be designated as a "micro-test." The filter area also has been reduced in proportion to the reduction in size of the sample. Since the method is quick, it can be used for a wide-spread educational program to control extraneous matter, or for a system of grading cheese according to the amount of foreign matter it contains, just as butter is graded according to the amount of salt it contains. H. Pyenson

- 294. Control of extraneous matter in Cheddar cheese.** J. P. JULIEN, R. DUMAIS, AND R. THIRODEAU. *Can. Dairy Ice Cream J.*, 26, 10: 34-36. Oct., 1947.

When the "micro-test" for sediment is used, it is possible to exercise control over extraneous matter in cheese on a large scale. A new method of preservation of cheese samples is described. It consists of keeping the samples in a sealed jar containing chloroform vapors. Chloroform proved to be an ideal preservative for this type of work, the samples remaining completely soluble and showing no signs of mold during long periods of time. H. Pyenson

- 295. Dichlorethyl ether in the control of cheese mites.** W. S. McLEOD AND R. W. BROWN. *Can. Dairy Ice Cream J.*, 27, 3: 80. March, 1948.

All cheese was removed from the room and dichlorethyl ether was applied at the rate of 1 lb. per 1000 cubic ft. The room was then closed and locked. On the following day the cheeses were washed and returned to the curing-room. It is advised that a respirator be worn while performing the fumigation. All mites were killed by a single application of dichlorethyl ether, and no reinfestation occurred during a period of 8 months. H. Pyenson

- 296. Retention of certain minerals and water-soluble vitamins in cheesemaking.** O. R. IRVINE, L. R. BRYANT, W. H. SPROULE, E. V. EVANS, H. S. JACKSON, A. COOK, AND W. M. JOHNSTONE. *Can. Dairy Ice Cream J.*, 26, 9: 35-40. Sept., 1947.

A study is reported of the extent to which the water-soluble vitamins, riboflavin and thiamine, and the minerals calcium and phosphorus are retained in cheesemaking. Of the original calcium present in the milk, about 61% was retained in raw-milk Cheddar cheese. Of the original phosphorus, about 53% was accounted for in the cheese. These values did not vary with season. About 23% of the riboflavin originally present in the milk was retained in the cheese. The results with pasteurized milk ("holder" and HTST methods) indicate that the heat treatment did not noticeably affect the retention of calcium. Cheese made from pasteurized

milk tended to retain slightly more of the phosphorus than did cheese made from raw milk. Pasteurization had no significant effect upon the retention of riboflavin when compared with the raw control.

The calcium, phosphorus and riboflavin contents of a limited number of batches of cream, cottage, brick and blue cheese were reported. Cream cheese contained 84.4 mg.% calcium, 86 mg.% phosphorus, and 280 mg. per 100 g. of riboflavin. Cottage cheese contained 85 mg.% calcium, 146 mg.% phosphorus and 288 mg. per 100 g. of riboflavin. In brick cheese, of the original nutrients present in the milk, 57.7% of the calcium, 58.7% of the phosphorus and 27.4% of the riboflavin were retained in the cheese. In blue cheese, the corresponding values for retention were: calcium 46.2%, phosphorus 43.3% and riboflavin 30.1%.

Cheddar cheese showed little or no loss of thiamine by either the holder or the "High-Short" process. The process of cheese manufacture caused no actual destruction of thiamine.

H. Pyenson

297. Report on sampling, fat and moisture in cheese. WILLIAM HORWITZ AND LILA KNUDSEN, Food and Drug Administration, Minneapolis, Minn. J. Assoc. Off. Agr. Chemists, 31, 2: 300-306. 1948.

A collaborative study was made of two methods for determining the moisture and fat contents of process American, rindless Cheddar and daisy Cheddar cheese. Moisture was determined by the official method and the force draft oven method, and the fat was determined by direct weighing of sample into a Mojonnier tube and by the official method. The average standard deviation of the official method for moisture determination was 0.23 and for the forced draft oven method 0.22. The average standard deviation for the Mojonnier tube method of determining fat was 0.44 and for the official method 0.48. Variation between samples of the same cheese at one laboratory was negligible, compared to the variation from one laboratory to another. The results indicated that the sample of shredded cheese sent to the collaborators was homogeneous and the differences obtained were not due to a difference between the samples sent to them.

F. J. Babel

298. Research problems in relation to Cheddar cheese quality. E. G. Hood. Can. Dairy Ice Cream J., 27, 4: 42. April, 1948.

The research projects under study are: (a) cause and control of such flavor defects as rancid, unclean and fruity; (b) mechanical openness; (c) control of extraneous matter in cheddar cheese; (d) clarification of milk in relation to quality; (e) starter problems; (f) bacteriophage in relation to cheesemaking; and (g) factors involved in the manufacture and storage of high quality cheese.

H. Pyenson

FOOD VALUE OF DAIRY PRODUCTS

299. Report on the phosphatase test in pasteurization of dairy products.

GEORGE P. SANDERS, Bureau of Dairy Industry, Washington, D. C.
J. Assoc. Offic. Agr. Chemists, 31, 2: 306-318. 1948.

Results indicated that the modified Kay-Graham procedure could not be adapted satisfactorily as an index of pasteurization in testing cheese. A description is given of the laboratory method of Sanders and Sager for testing various dairy products to determine the adequacy of pasteurization. The method includes modifications needed to produce uniformly quantitative results under fixed conditions in applying the test to various common varieties of cheese, fluid milk, cream, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk and cheese whey.

Phosphatase activity caused by microorganisms was not encountered in any fresh or reasonably fresh products. It was encountered in some samples of old butter, old cream, surfaces of soft and semi-soft ripened cheeses and in several specific cultures of microorganisms.

The substrate *p*-nitrophenyl phosphate decomposed relatively rapidly under the influence of heat when the controls and tests were heated after incubation. Even less precision was obtained with phenolphthalein phosphate than *p*-nitrophenyl phosphate.

The Sanders-Sager method was recommended by the Associate Referee as the official method for testing fluid milk and cream, cheddar type cheese and soft unripened cheeses for the index of adequacy of pasteurization. Also, he recommended that this method be made tentative for other types of cheese, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, cheese whey and concentrated milk products. It was recommended that the present phosphatase test for pasteurization be dropped. F. J. Babel

300. Combination of formaldehyde with casein. A. P. SWAIN, ELSIE L. KOKES, N. J. HIPP, J. L. WOOD, AND R. W. JACKSON, Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Ind. Eng. Chem., 40, 3: 465-469. March, 1948.

Graphs are presented to show the effects of concentration of formaldehyde, pH, time, and temperature on the amount of recoverable formaldehyde remaining in combination with casein after exhaustive washing of the reaction product with distilled water. The results are compared with related data of other investigators and are discussed in terms of possible reactions of various structural units in the protein. The analytical procedures employed for distillation and titration of recoverable formaldehyde

were extensively studied and improved. Experiments also are described that show appreciable conversion of formaldehyde to the nonrecoverable form in the presence of casein at 100° C. and above. B. H. Webb

301. Vitamin A and carotenoids in the blood serum of dairy cattle. Chemical methods for determination. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES. Kansas Agr. Expt. Sta., Manhattan. Analyt. Chem., 20, 3: 230-233. March, 1948.

Four methods for the determination of vitamin A and carotenoids were compared. When cows received large amounts of vitamin A supplements, the results of the vitamin A determination of blood serum were too low if, without preliminary saponification, a method was employed that utilized carotene precipitation for the removal of interfering substances. Certain components of blood serum in addition to carotene interfered with the determination of vitamin A by the Carr-Price reaction. The interfering substances were susceptible to saponification and to milk oxidation.

B. H. Webb

302. Factors affecting the keeping quality of dried milk powder. R. A. CHAPMAN. Can. Dairy Ice Cream J., 27, 4: 45-46. April, 1948.

Good quality milk powders can be stored satisfactorily for periods up to 2-3 years if they are gas packed in an atmosphere containing 3% or less of oxygen. The solubility will not be impaired if the moisture content is kept below 3%. Ethyl gallate has been found very effective, either independently or in conjunction with a pre-heat treatment, in inhibiting oxidative deterioration.

H. Pyenson

303. Experimental enterococcal food poisoning in man. A. G. OSLER, L. BUCHBINDER, AND G. I. STEFFEN. Proc. Soc. Exptl. Biol. Med., 67: 456-459. 1948.

Symptoms of acute gastric or intestinal disturbance or both were produced in 6, or possible 7, of 26 human volunteers who consumed egg salad, custard, or milk in which single strains of *Streptococcus fecalis* had grown for 5 hrs. Attempts to produce similar symptoms in man with 20-hr. cultures grown in milk or in infusion broth, were unsuccessful. I. Peters

304. Mineral metabolism studies in dairy cattle. III. Manganese metabolism in the lactating bovine. J. THOMAS REID AND GEORGE M. WARD. N. J. Agr. Expt. Sta., Sussex. J. Nutrition, 35, 5: 591-596. May 10, 1948.

With manganese intakes ranging from 622.4 to 1325.6 mg. daily, cows

retained about 154 mg. daily during the first 5 months of lactation. The fecal elimination of manganese was proportional to intakes within the above range. Manganese in the form of manganese sulfate was utilized as well as the manganese of feed.

R. K. Waugh

- 305. The nutritional value of cheese to the consumer as compared with the price of milk.** A. L. GIBSON. *Can. Dairy Ice Cream J.*, 27, 4: 62-66. April, 1948.

A method for calculating the nutritional value of cheese to the consumer on the basis of the retail price of milk is given in detail. The constituents of milk and cheese are converted to a common unit basis. The unit used is lactose. Using this unit system, tables are given comparing the value of 1 lb. of cheese to the consumer as compared with the retail price of milk. This method offers a sound system of cost accounting. H. Pyenson

ICE CREAM

- 306. Emulsifying and stabilizing agents for ice cream.** LAWRENCE L. LITTLE, E. F. Drew Co., Boonton, N. J. *Milk Plant Monthly*, 37, 6: 42-48, 50. 1948.

The author describes fully the role of the basic stabilizers, such as gelatin, sodium alginate, vegetable gums and cellulose gum in ice cream as well as emulsifying agents. The emulsifying agent discussed is restricted to the fat-soluble type which is a derivative of a natural fat in which the fat has been modified so as to form a water soluble group in the molecule. The hydrophilic triglyceride added to the ice cream mix upon homogenization is oriented so that the water soluble groups are at the surface of the fat globules, thus forming a water soluble coating on the surface of the fat globules binding a film of water around the fat and holding it as water of hydration. In this way the hydrophilic triglyceride has stabilizing properties in that it (a) reduces the amount of water that will be converted into ice when the mix is frozen and hardened, (b) the hydrated fat globules function as hydrated colloid particles in deflecting the growth of large ice crystals into more numerous and smaller crystals and (c) the fat globules, hydrated as a result of the action of the hydrophilic triglyceride, counteract the dehydrating effects of freezing upon the stabilizer and milk proteins. Probably the most important function of an emulsifying agent in ice cream mix is its ability to increase the cohesion of the mix as a result of its property of holding a film of bound water around fat globules and the ability to retain this bound water through the freezing and hardening operations.

G. M. Trout

307. **Emulsifiers—how they improve ice cream.** H. L. CASLER, Germantown Manufacturing Co., Philadelphia, Pa. *Ice Cream Rev.*, 31, 10: 52, 54. May, 1948.

Emulsifiers supplied to the ice cream industry belong to that class of chemical compounds known as "esters." These are combinations of long chain fatty acids such as palmitic, stearic or oleic, and a higher alcohol such as glycerol or sorbitol. They have an affinity for both water and fat. The fatty acid end of the molecule is soluble in fat, whereas the alcohol is soluble in water. Furthermore, they are powerful surface active agents, i.e., they move to any interface where fat and water come together and greatly reduce surface tension, which is the force that tends to cause fat to form the largest possible masses. The homogenizer easily can reduce the butterfat to sub-microscopic globules with the surface tension force removed. The result is a finer textured ice cream. Finally, the esters spread out over all interfaces until they entirely surround the tiny fat globules, thus forming a protective film which in conjunction with the stabilizer prevents clumping of the fat globules. Much the same action is believed to occur around the air cells of the finished ice cream, thus improving overrun and combating shrinkage. Since the dispersing and protective actions have no connection with low temperature, better and smoother melt-downs usually result.

Emulsifiers do not take the place of a stabilizer. When a combination emulsifier and stabilizer is purchased, the ratio between the two usually is correct. When emulsifiers are purchased by themselves, the usual amount of stabilizer should be used and the optimum amount of emulsifier should be determined by trial. Emulsifiers will give results only in a pasteurized and homogenized mix. Only at pasteurizing temperatures does the complete and intimate contact take place which is necessary for surface action; the resulting lowered surface tension merely is an aid to better homogenization. It is the homogenizer and emulsifier together which improves the emulsion.

Use of emulsifiers in the margarine industry dates back to 1920, and they are commonly used in the baking and confectionery industries. They are considered safe and have been proved to be non-toxic. In the purchase of an emulsifier for use in ice cream, it is important that a product be selected which is fully edible and which is completely free from objectionable flavors or odors.

W. J. Caulfield

308. **Mix stabilizers and whipping agents in making ice cream.** P. H. TRACY. *Can. Dairy Ice Cream J.*, 26, 10: 42-46. Oct., 1947.

The sources, manufacture and characteristics of the following mix stabilizers and whipping agent are discussed: (a) gelatin; (b) Irish moss;

(c) carob bean; (d) pectin; (e) Dariloid; (f) quince seed extract; (g) gums; (h) sodium carboxymethylcellulose and (i) emulsifying agents.

H. Pyenson

309. Ice cream shrinkage. H. A. BENDIXEN. *Can. Dairy Ice Cream J.*, 27, 4: 54-60. April, 1948.

The following precautions would be helpful in guarding against ice cream shrinkage: (a) avoid freezing the ice cream too stiff in the continuous freezer; (b) avoid extreme temperature changes or heat shocking; (c) use high-quality low-acid dairy products to prevent destabilization of the proteins; (d) avoid an excessively high sugar content and especially a high dextrose content; and (e) avoid the use of unparaffined cartons or cans, banging of the packages, and excessive air circulation directly over the ice cream in the storage room.

H. Pyenson

310. Quality in ice cream. E. L. WALKER. *Can. Dairy Ice Cream J.*, 26, 9: 31. Sept., 1947.

As high a quality of ice cream can be made with low fat content as with high fat content. There is usually a greater per capita consumption of ice cream where a high quality of ice cream is produced. Checking every product of ice cream for test, taste, color, flavor and texture before and after it is manufactured and before it leaves the plant should be a standard procedure.

H. Pyenson

311. Formula for the future. W. GRIFFITH. *Ice Cream Trade J.*, 44, 5: 42, 43, 92-95. May, 1948.

With ice cream sales going down in the face of increasing national income, the author makes the following 4 suggestions for turning ice cream sales upward: Make a lower butterfat, quality product; go after the packaged market; clean up the dealer's store; and get Johnny's and Mary's nickel.

W. H. Martin

312. High temperature-short time pasteurization of ice cream mix. C. M. MINTHORN, Chester Dairy Supply Co. *Ice Cream Trade J.*, 44, 5: 70, 70B, 99, 100. May, 1948.

Lack of suitable equipment has delayed the general use of high-temperature short-time pasteurization of ice cream mix. In plants where no condensing is done and only concentrated products are used, the heating of the mix must be done in two stages. The ingredients are placed in a mixing vat, pumped through a tubular heater, and heated to 125° F. before the final pasteurization treatment. In plants using raw products, and

where the condensing operation is a part of the mix-making system, the ingredients are mixed and pumped through a tubular heater before they pass to the vacuum pan for concentration to about 40% solids; this mixture is pumped into measuring tanks before final pasteurization. In the final operation the mix is picked up from the balance tanks with a centrifugal pump and pumped through a filter and through the first 16 tubes of another heater, where the temperature is raised from 120 to 160° F. From here the mix goes to 2 homogenizers, one of 1,250-gallon capacity and the other 400-gallon capacity per hour, where the mix is homogenized at 160° F. The discharges from these 2 homogenizers converge into a return line and go back to the heater, where the temperature is raised to 176° F. through the last 8 tubes of the heater. From this point, it goes to the holding tube, where it is held 22 seconds before going to the ice cream mix cooler. In this process the mix is heated to 160° F. before it goes to the homogenizers, which are metering pumps. The centrifugal pump is of slightly greater capacity than the homogenizers so that there is a continuous pressure in the suction side of the homogenizer so that no air is incorporated in the mix.

W. H. Martin

- 313. Latest developments in hardening ice cream.** HARRY BITTERS. *Can. Dairy Ice Cream J.*, 26, 10: 66. Oct., 1947.

With the new method of filling direct in the carton, it is necessary to harden at a much faster pace to keep up with the capacity of the freezer. A freezing tunnel with a temperature of at least -40° F. with a blast of air circulated over the conveyor carrying the packages works satisfactorily. Four 30-in. high-speed fans can harden pint packages in 70 minutes at a capacity of 375 gallons per hr. The conveyor has a variable speed drive. The disadvantages are: (a) the oil in bearings and transmission is very likely to solidify; (b) leaks cause loss of refrigeration; (c) the formation of ice around the conveyor interferes with the operation. Defrosting is best accomplished by the use of hot gas.

H. Pyenson

- 314. Chocolate ice cream.** R. A. SIMONET, Robert A. Johnston Co., Milwaukee, Wis. *Ice Cream Rev.*, 31, 10: 56, 58, 59. May, 1948.

The ideal combination for flavoring chocolate ice cream is a mixture of equal parts of chocolate liquor and cocoa. Such a mixture will have a fat content of 34% and a melting point of 92° F. The chocolate mixture plus 2% additional sugar should be incorporated into the mix at the pasteurizer. When homogenized at 1,500-2,000 lb. pressure, the possibility of specks in the finished ice cream is eliminated. Overnight aging will enhance a mellow chocolate flavor free of sharpness.

Straight chocolate liquor is not recommended for use in ice cream be-

cause it does not carry sufficient color and cannot be conveniently prepared as a paste for addition at the freezer. Cocoa does not carry enough of the high melting cocoa fat and the flavor does not remain long enough in the mouth. The proposed mixture with a melting point of 92° F. will, on the other hand, linger on the taste buds of the mouth long after the ice cream has been swallowed.

Caution is urged against the use of a chocolate product which is too acid or alkaline. If a too highly dutched product is used, the ice cream is apt to have a dull muddy appearance and may develop greenish spots if stored in poorly tinned metal cans without paper liners. Procedures are outlined for the production of variegated chocolate ice cream with both the batch and continuous freezers. It is strongly recommended that a weekly tasting panel be set up to examine all chocolate flavored ice creams being offered for sale and to determine what adjustments, if any, should be made to satisfy local market demands. W. J. Caulfield

315. Frozen berry purees and their application in the dairy industry.

E. H. WIEGAND. *Can. Dairy Ice Cream J.*, 26, 9: 41-43. Sept., 1947.

Soft ripe fruit is used in the manufacture of purees to obtain an excellent product of high flavor. Hard fruit lacks flavor and may produce a bitter puree. The two types of purees suitable for ices and ice cream are the simple frozen puree and the pectinized puree. The fruit should be pulped at a low temperature (35° F.) to prevent oxidation. Thorough deaeration is essential to produce purees of better keeping quality. The fruit is cleaned, peeled, if necessary, crushed and frozen in barrels or enamel-lined cans. Simple purees are suitable for sherbets, ices, Velva fruit, and soda fountain flavors and pectinized purees for ribbon or ripple ice cream. Formulas are given for the preparation of pectinized purees from various berries, pears, nectarine, apricot, Velva fruit, high acid-low pectin content fruits and low acid-high pectin content fruits. H. Pyenson

316. Evaluating the flavor of ice cream. D. V. JOSEPHSON. *Can. Dairy Ice Cream J.*, 27, 5: 50-56. May, 1948.

Apart from individual preferences, the composition of ice cream is sometimes dictated by the economic and racial status of a community. The total flavor response is the result of the 3 separate functions of the taste mechanism, taste, touch and smell. All people do not have the same degree of development or refinement in their taste functions. The over-all flavor of ice cream is a delicate blend of sweet and salty taste responses coupled with the olfactory response characteristic of the particular flavor in question. The tactual taste receptors on the tongue contribute to the over-all flavor by recording the texture and "feel" sensation in the mouth.

Some of the factors that influence the efficiency of taste work are sensitivities and patterns of taste response of the judges; scoring ranges are too wide and do not permit an accurate or tangible evaluation of the product; a key or dominant figure whose scores involuntarily become the standard for the group; choosing a time for judging samples; too many samples are included in each judging session; and taste panels evaluate ice cream in terms of their own personal opinion which may not necessarily represent the average consumer. Some suggestions for improving tasting techniques are as follows: (a) calibrate each member of the taste panel and determine their relative sensitivities or thresholds to basic, abnormal and deteriorative flavor qualities; (b) a maximum range of 5 to 6 points should be employed; (c) every judge should have complete freedom in expressing his judgments; (d) establish a standard and uniform terminology for describing the flavors and textures of ice cream; (e) have ample time for judging; (f) judge in room where there are no disturbances or noise; (g) evaluate products in terms of consumer's preference; (h) delete identification marks on packages; and (i) a member of the taste panel should not set up the samples.

H. Pyenson

317. A consumer taste-test panel. ANONYMOUS. *Ice Cream Trade J.*, 44, 5: 34-35, 95, 96. May, 1948.

A consumer clinic which regularly tests ice cream has been set up by a dairy laboratory at Scranton, Pa. The tasters who form the panel are chosen from among professional and business people, housewives and nutritionists; participation is by invitation only. "Judgment sheets" calling for scores on appearance, body, texture, and flavor, along with other comments or criticisms, are used by the testers. These independent taste panels provide a guide for the manufacturer to use in the determination of the quality of his ice cream. Opinions of these impartial judges can help to wipe out many fallacies that exist in the consumer's mind. A copy of the completed clinical report is furnished to the ice cream manufacturer subscribing to the clinic, and he also receives a laboratory and bacterial analysis showing the percentage of fat, total solids, acidity, homogenization index and standard plate and coliform counts on the ice cream.

W. H. Martin

318. Stick novelties operation in medium-sized plant. T. G. MUNROE. *Can. Dairy Ice Cream J.*, 27, 5: 66-70. May, 1948.

All products, equipment and methods were standardized after the early patent rights were acquired, and this material was controlled by a single management. In order to produce stick novelties successfully, it is necessary to have proper equipment (brine tank), filling molds, sticking by ma-

chine, defrosting of molds quickly, chill or drying tunnel and dipping, bagging, and packing equipment. Some of the most important points in stick novelties operation are keeping the operations up to date with the latest money-saving equipment of proper capacities, streamline the operations, prevent waste of material, cartons, bags, sticks and coating, and use effective advertising in the dealers' stores.

H. Pynson

- 319. Trends in the ice cream business.** J. L. DOLPHIN. *Can. Dairy Ice Cream J.*, 27, 4: 32-36. April, 1948.

Trends in the manufacture and sale of ice cream and suggestions for developing marketing of ice cream are discussed. The subjects reviewed are batch freezers, continuous freezers, shrinkage, brick ice cream, overrun, deep freeze boxes, packed ice cream, advantages of pint packages, and advantages of round package.

H. Pynson

- 320. New Jersey ice cream men campaign against sale of ice cream by weight.** ANONYMOUS. *Ice Cream Rev.*, 31, 10: 74, 76, 78, 80, 82. May, 1948.

Some of the reasons advanced against the sale of ice cream by weight are: (a) It is impossible to produce ice cream with a uniform weight per gallon. The weight per unit value is not only influenced by the per cent overrun, but by the composition of mix, the added flavors, etc. Since no tolerance is allowed in the measurement of products sold by weight under the New Jersey law, each package would have to be weighed individually and have its exact weight stamped on the container. This would necessitate extra labor and inventory records would be more complicated. (b) The weight system would reduce the efficiency of the individual truck driver. More time would be involved in checking out his load at the plant and in billing dealers for goods received. (c) Extra costs involved in producing ice cream by weight would of necessity have to be passed on to the consumer. (d) Sale of ice cream by weight by some 15,000 retailers in New Jersey would necessitate the purchase of special scales and would slow up service. (e) Sale of ice cream by weight does not mean the consumer will receive more value for his money. On the contrary, the practice probably would lead to the production of an ice cream with a minimum fat content, with the weight increased by the addition of extra sugar solids. (f) Retailers and the public are protected against receiving ice cream with excessive amounts of overrun by the present New Jersey law, which requires that ice cream be manufactured with a minimum weight of 4.5 lb. per gallon. (g) Public enforcement of fair measure is much more feasible on a volume basis than on a weight basis. The consumer can see when a container is not properly filled, but few will have scales to check the

weight of the product. (j) Sale of ice cream by weight would lead to untidiness and carelessness in weighing out the various packages or servings at soda fountains. (k) The sale of ice cream by weight has not proved successful where it has been tried. The experiment of selling ice cream by weight lasted for less than a year in Los Angeles, and was discontinued at the request of the druggists association which had sponsored the move. (l) The majority of dealers in New Jersey oppose the sale of ice cream by weight. (m) There is no great public demand for the sale of ice cream by weight. Despite the fact that the New Jersey law now permits the sale of ice cream by weight, only a handful of dealers have availed themselves of this privilege.

The ice cream manufacturers of New Jersey make it clear that they are not opposed to a law against the sale of ice cream by weight if a just, workable law could be devised. They do oppose a law which would work a hardship on the public and discriminate against the manufacturers and dealers.

W. J. Caulfield

MILK

321. **What constitutes quality in milk.** G. M. Trout. *Can. Dairy Ice Cream J.*, 26, 9: 32-34. Sept., 1947.

Different quality factors are emphasized by various groups. The various groups to consider are the consumer, the producer, the processor and distributor, the bureau of standards inspector and the milk sanitarian or health officer. The following factors enter into a definition of quality milk: (a) cream line; (b) appearance; (c) safety; (d) cleanliness; (e) good flavor; (f) keeping quality; (g) nutritive quality; (h) low bacteria count; (i) esthetic background of production; (j) composition; and (k) miscellaneous, such as freedom from adulterants, no watering, etc.

H. Pyenson

322. **The time factor in high-temperature short-time pasteurizing.** H. B. Robinson and C. M. Moss, U. S. Public Health Service, Dist. 1., New York City. *J. Milk and Food Technol.*, 11, 1: 44-51. Jan.-Feb., 1948.

The U. S. Public Health Service Milk Ordinance and many state and local regulations specify 160° F. for 15 seconds for high-temperature short-time pasteurization. The specifications include a safety zone of at least 9 seconds at 160° F. and conversely of at least 5° F. if the time is 15 seconds. There appears to be no need for increasing either the time or temperature beyond those margins established by the fluctuations of the pasteurizer controls.

Large errors are inevitable where water runs are used in timing high-temperature short-time units. A variation of 26.7% or more was noted when water and milk were delivered to the same pump. Therefore milk run tests should be used in timing. Both the volume delivered and temperature fluctuation technics appear to have application in extending water run tests to milk by empirical correction.

H. H. Weiser

323. Homogenized milk problems. P. H. TRACY, Univ. of Ill., Urbana. *Milk Plant Monthly*, 37, 6: 58-68. 1948.

Problems encountered with homogenization of milk include sufficient breakdown of the fat globules to prevent fat rising, curd tension reduction, prevention of sediment formation, maintenance of low bacterial content of the homogenized milk, testing for fat by the Babcock method, curdling during cookery and flavor defects—particularly rancid and sunlight flavors. These defects can be overcome by carrying out the homogenization process effectively through using sufficient pressure, maintaining proper temperatures and having valves in good condition. Many data are presented showing the effects of numerous processes on sedimentation in homogenized milk. If the cell content of non-homogenized milk is less than 200,000 per ml., likely the homogenized milk will not show enough sediment to be of any consequence. A low bacterial count can be obtained by washing and sterilizing the homogenizer after each day's use according to prescribed procedures. Especial attention must be paid to keeping homogenized milk out of sunlight if the sunshine flavor is to be prevented.

G. M. Trout

324. Problems on quality milk production. C. D. MACKENZIE. *Can. Dairy Ice Cream J.*, 27, 4: 39-40. April, 1948.

The problems relating to high quality milk may be divided into those dealing with cleanliness of milk, such as disease and management, and those dealing with the nutritive value of the product as affected by breeding and feeding. The diseases important to eliminate for high quality milk production are mastitis, Bang's disease and tuberculosis. The relation of management, sanitation, breeding and feeding is discussed in relation to quality milk production.

H. Pyenson

325. Should we have a simple standard of milk quality. G. M. TROUT. *Can. Dairy Ice Cream J.*, 26, 10: 60-62. Oct., 1947.

Recent advances in production, distribution and public health point more and more toward the realization of a simple standard of quality for milk.

H. Pyenson

- 326. Prospective milk production and consumption in Canada.** B. L. CAMPBELL. *Can. Dairy Ice Cream J.*, 27, 3: 68-72. March, 1948.

A study was made of prospective trends based on dairy, agricultural and business statistics and an interpretation of these statistics are given.

H. Pyenson

- 327. Sanitation and its control.** G. E. STANLEY. *Can. Dairy Ice Cream J.*, 26, 10: 38-40. Oct., 1947.

There has been a tremendous expansion in the consumption of milk during recent years, but very little has been done to improve the sanitary quality of the raw product. The article discusses bacterial counts, mastitis, bacteriological surveys and laboratory methods of control.

H. Pyenson

- 328. Farm inspection for better milk supply.** A. E. BERRY. *Can. Dairy Ice Cream J.*, 27, 2: 64-70. Feb., 1948.

Looking back over a period of 30 years in Canada, the article discusses: (a) control of disease; (b) accomplishments of pasteurization; (c) legislation and administration; (d) new health units; (e) qualification of personnel; (f) farm inspection by veterinarians; (g) bacterial standards; (h) standard for pasteurized milks; (i) factors in farm inspection; (j) cleaning of utensils and (k) cooperation of distributors.

H. Pyenson

SANITATION AND CLEANSING

- 329. Bottle washing problems.** D. H. JACOBSON. *Can. Dairy Ice Cream J.*, 26, 9: 44-50. Sept., 1947.

Efficient sterilization of bottles is obtained by the use of proper time, temperature and caustic strength. The problems in soaker bottle washing are: (a) efficient sterilization; (b) complete cleaning and bright appearance; (c) prevention of etching of glass or color labelling; (d) prevention of scale on the washer; and (e) smooth mechanical operation or lubrication. Methods of obtaining satisfactory results are discussed.

H. Pyenson

- 330. Can washing in creameries and milk manufacturing plants.** P. J. BOGAERTS. *Can. Dairy Ice Cream J.*, 27, 3: 74-78. March, 1948.

Milk and cream cans must be sent out from the creamery or milk plant to the farm in an absolutely sterile condition whether the cleaning operations are conducted by hand or by machine can washing. The methods of cleaning mechanical washers are given in detail.

H. Pyenson

- 331. Cleaning and sterilizing of dairy equipment.** J. S. GEORGE. *Can. Dairy Ice Cream J.*, 26, 9: 52-53. Sept., 1947.

The old standard cleaning materials are discussed giving new applications for their use. These materials discussed are the carbonates or soda cleaners, silicates, phosphates, complex phosphates, caustic soda, washing compounds and acid cleaners. The new materials developed are the synthetic detergents and the quaternary ammonium compounds.

H. Pyenson

- 332. A rapid field test for quaternary ammonium salts used in germicides.** R. F. BROOKS AND G. F. HUCKER, New York State Experiment Station, Geneva, N. Y. *J. Milk and Food Technol.*, 11, 3: 136-138. May-June, 1948.

A rapid routine test for checking quaternary ammonium solutions has been proposed. It is not intended to be an accurate quantitative method. The method is based on the principle that an excess of a mixture of quaternary salts and ethylene dichloride in the presence of certain anionic indicators in acid solution produces a yellow-green color in the ethylene dichloride layer of the mixture. This gives a sharp contrast with the blue-violet color of the aqueous layer, making the end point more easily visible. Approximately 1 ml. of ethylene dichloride is added to 1 ml. of the quaternary ammonium solution in a small bore test tube. The tube is inverted to facilitate mixing. Bromphenol blue indicator (buffered at pH 4.5 to 4.8) is added (one drop to approx. 0.05 ml.) The contents of the tube are mixed by inverting after each addition of indicator and allowing the layers to separate before adding more indicator. The number of drops of indicator required to give a permanent yellow-green color in the ethylene dichloride (lower) layer as compared to a blue-violet color in the aqueous (upper) layer, indicates the concentration of quaternary ammonium solution.

H. H. Weiser

- 333. Quaternary ammonium and hypochlorite solutions for sanitizing dairy utensils and equipment.** C. K. JOHNS. *Can. Dairy Ice Cream J.*, 27, 3: 27-29. March, 1948.

The quaternary ammonium germicides have many desirable properties which make them suitable for use in the sanitizing of dairy utensils and equipment. In general, they were slightly more effective than the hypochlorites against gram-positive organisms; against gram-negative organisms, the reverse held true. Cheese starter organisms were killed more readily by hypochlorites. The type of water used to prepare dilutions of the two types of germicide appeared to have some influence. Tap water solutions of "QA" (one of the quaternaries) were more sensitive to added skim

milk than were distilled water solutions, while hypochlorites showed little difference. Hypochlorites were much less sensitive to added skim milk than is usually believed. While the quaternaries showed some response to favorable adjustments in pH and temperature of solution, the response was slight compared with that shown by hypochlorite. As preservatives in milk, quaternaries are far less effective than formaldehyde. H. Pyenson

334. **Water treatment and the use of chemicals in the creamery.** L. R. BRYANT. *Can. Dairy Ice Cream J.*, 27, 4: 27-30. April, 1948.

Lack of use, or improper use of water conditioners, water softeners, cleaning compounds and sterilizing chemicals can produce large losses through breakdown and replacement of equipment, lowered efficiency, inadequate cleaning and sterilizing of both equipment and containers and consequent lowering of product quality. The 3 main problems connected with the water supply in a creamery are: (a) scale on the surface of any heat exchange type of equipment; (b) corrosive action of some water; (c) source of water used for washing butter. The following methods of treating water are discussed: (a) silicate treatment; (b) chromate treatment; (c) complex phosphate threshold treatment; (d) water softening; (e) alkali cleaners; and (f) acid cleaners. H. Pyenson

335. **Testing equipment as a practical aid to efficient sanitation.** LEE H. MINOR. *Can. Dairy Ice Cream J.*, 26, 10: 48-50. Oct., 1947.

The service testing equipment that can be used readily to aid efficient sanitation includes: (a) a germicidal test kit for determining the amount of available chlorine in parts per million in germicidal rinse solutions; (b) a film tester for determining the source of film on equipment; (d) a control meter which automatically registers on a dial the concentration of a cleaning solution by percentage; (d) an alkometer for registering on a dial the percentage of caustic concentrations in the washing solution; (e) a titration test kit for making chemical tests and titrations; (f) germicidal test papers for quickly checking p.p.m. of available chlorine; and (g) alkalacid test paper for checking a solution to determine whether acid or alkaline.

H. Pyenson

MISCELLANEOUS

336. **The effect of inanition on mammary-gland development and lactation.** J. F. SYKES, T. R. WRENN, AND S. R. HALL, Bureau of Dairy Industry, USDA, Beltsville, Md. *J. Nutrition*, 35, 4: 467-476. April 10, 1948.

One group of rats was fed from weaning through pregnancy with feed

intake limited to 70% of that received by controls. At parturition, one-half of the rats in each group were sacrificed. Mammary gland weight at parturition averaged 3.34 g. for the control group and 1.87 g. for the restricted diet groups. Following parturition, all remaining rats were full fed, and allowed to suckle litters for 21 days, and then sacrificed. At the end of 21 days, litters of the control group had gained an average of 163.8 g., the litters of rats restricted during pregnancy 198.3 g. At the end of the suckling period average mammary gland weight for the control group was 283.5 g. and for the group restricted during pregnancy 220.4 g.

R. K. Waugh

- 337. Value of research to dairy industry.** W. H. Cook. *Can. Dairy Ice Cream J.*, 26, 9: 27-30. Sept., 1947.

Research is any effort designed to yield something new, whether new knowledge, new processes or new products. Canadian industry might increase its expenditures for research to bring them more in line with the research expenditures made by industries in other countries. The article deals with (a) the cost of research, including some suggestions as to how the limited funds might be used to the best advantage; (b) the specific projects of the research program; and (c) the returns to be expected from these research expenditures.

H. Pyenson

- 338. Apparatus for homogenizing mixed liquid ingredients.** J. B. McFADDEN. (Assigned to United Dairy Equipment Co.) U. S. Patent 2,441,711, May 18, 1948 (3 claims). *Official Gaz. U. S. Pat. Office*, 610, 3: 669. 1948.

Two discs, rotating within a casing and in opposite directions, have radially spaced concentric projecting blades, so disposed that intimate mixing of liquid ingredients is effected. Emulsification, comminution and mixing are accomplished by impacting the product against the sharp cutting edge of each blade, the product moving from one blade to another by centrifugal force.

R. Whitaker

- 339. Survey indicates continued trend toward converting boilers to oil or gas.** ANONYMOUS. *Ice Cream Rev.*, 31, 10: 50. May, 1948.

A survey made in the South Atlantic, South Central, Mountain, and Pacific States revealed that 75.6% of the 243 ice cream firms replying to the questionnaire are already using gas or oil-fired boilers. Of the 7.5% of firms reporting they were contemplating a change in boiler fuels, 45.5% indicated they were planning to shift from coal to oil, 13.6% from coal to gas, and 9% indicated they planned to shift from oil to coal (because of greater economy and because of the uncertainty of oil deliveries). The ice cream industry has converted from coal to oil or gas to a greater extent than

have other branches of the dairy industry. Only 56.6% of the milk dealers and 34.6% of the creameries surveyed were using oil or gas.

Of 276 ice cream manufacturers replying to a questionnaire on the number of boilers, boiler capacity, and loads in their respective plants, over half of the plants had boiler capacities of less than 40 h.p., with the number of boilers per plant averaging 1.9. The boiler loads of over 75% of the plants varied from hour to hour and from day to day, whereas 24.6% of the plants indicated they operated with steady boiler loads. In over 60% of the plants, 90% or more of the steam generated was used for processing. Steam used for heating the building amounted to only 10% of the total generated in about half the plants and was less than 30% in over 90% of the plants.

The reasons most frequently given for conversion from coal to oil or gas were: cleaner, saves labor, convenient, economical and efficient. Before any contemplated changes from coal to oil are made, ice cream manufacturers are cautioned that they should first obtain the assurance of some responsible and reputable company that it will take care of their oil requirements on an equal basis in the amount estimated they will require.

W. J. Caulfield

340. New developments in the field of refrigeration. W. H. MARTIN.
Can. Dairy Ice Cream J., 26, 9: 60-66. Sept., 1947.

It is desirable to operate the refrigerating equipment as efficiently and economically as possible to maintain a high overall plant efficiency. Important points to remember are: (a) operate with as high an evaporating temperature as possible; (b) the coldest water available should be used for condensing purposes at a rate of about 3 U. S. gallons per ton-minute; (c) remove air or foreign gas from the system; (d) operate with as low discharge pressure and as high suction pressure as possible; (e) save refrigeration by regenerative processes and by using water. Other details discussed are evaporative condensers, moisture in the system and refrigeration controls.

H. Pyenson

341. Family consumption and dairy products. ANNA M. SPEERS. *Can. Dairy Ice Cream J.*, 27, 5: 42-48. May, 1948.

The consumption of dairy products depends upon national income, educational efforts, food expenditure, number of adults and number of children in the household. Milk utilization is a factor in the amount of milk used. The consumer wants safe milk, milk labelled as to grade, and square containers, preferably glass. In a sample group study of 70 families that average \$1,820 per year, as prices rose, they began to stop buying clothing, household furnishings and utensils, and decreased expenditures on recreational gifts. The largest portion of the budgets of these families is today being spent on food, shelter, fuel and light.

H. Pyenson

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

342. **Advances in enzymology and related subjects of biochemistry, vol. VIII.** Edited by F. F. NORD. 538 pp. \$8.00. Interscience Publishers, Inc., New York, N. Y. 1948.

The 8 chapters in this volume are: Functioning of the cytoplasm; Quantitative studies on complement; Dehydropeptidases; Antifatty-liver factor of the pancreas—present status; Alkaloid biogenesis; Certain aspects of the microbiological degradation of cellulose; Synthesis of lipides; The biochemistry of fatty acid catabolism; Lipoxidase and the autoxidation of unsaturated fatty acids; and Enzymes of snake venom and their biological significance. Of the 14 authors, 5 are resident in foreign countries, thus contributing to the international flavor of the presentation. The presentation for each subject appears to be adequate and critical. The material is well-organized and well-indexed. This volume contains a cumulative index for all 8 volumes which have appeared to date. This is a valuable contribution in the struggle to summarize the increasing mass of scientific literature in forms which permit scientists to keep informed in areas outside of their limited specialties.

F. E. NELSON

343. **Manual of veterinary bacteriology.** RAYMOND A. KELSER AND HARRY W. SCHOENING. 5th edn. 767 pp. \$6.50. The Williams and Wilkins Co., Baltimore, Md. 1948.

The current edition of this book is a mixture of the up-to-date and the archaic in its field, the former predominating in some sections and the latter in others. Much material on specialized technics of value to bacteriologists handling pathogenic microorganisms, some of it not available from other common sources, is presented. The sections on general bacteriology and infection and immunity are the most in need of modernization. To mention a few specific examples, the particulate nature of bacteriophages and viruses is discussed in terms of diffusion experiments of 12–15 years ago, with no mention made of the numerous electron microscope micrographs and studies of the past 7 years. The different forms of penicillin receive no consideration. Except for two references to textbooks published in the middle thirties only one reference to work published after 1903 is given in the unexpectedly brief chapter on infection and immunity. Although the sixth edition of *Bergey's Manual* was published several months earlier

by the same publisher and the revised classification to be used had been publicized in some detail long before that, the classification employed, and thus the organization of the largest part of the book, is based upon the fifth edition of *Bergey*. In the chapter on milk and water bacteriology, bases for interpretation of results are lacking. Standards for coliform bacteria in either milk or water are not presented. Five lines, including one reference to an article published in 1935, are devoted to the phosphatase test, which might very well have occupied at least the half page devoted to the relatively unimportant color changes which bacteria may cause in milk. The illustrations are neither qualitatively nor quantitatively what one expects in a book published in 1948. The discussions on the microbiology of diseases which affect cattle are the portions which probably would be most useful to those in the dairy industry. F. E. NELSON

344. **Bacteriology. A textbook of microorganisms.** F. W. TANNER AND F. W. TANNER, JR. 4th edn. 625 pp. \$4.50. John Wiley & Sons, Inc., New York, N. Y. 1948.

An appreciable amount of new material has been added in various places in the revision of this text, which treats bacteriology as such instead of as a branch of the medical profession. A tendency for more explanation and somewhat more concise organization than in earlier editions is apparent. The book is a good source of general information on microbiology.

Despite the generally favorable impression, several shortcomings which one hardly expects in a revision dated 1948 were encountered. The illustrations, on the whole, leave quite a bit to be desired, as many of them are either completely out of date or much inferior to what could be obtained without undue effort. By October 1947, when this book apparently went to press, the outline of the classification system used in the sixth edition of *Bergey's Manual of Determinative Bacteriology* had been publicized quite widely, so there seems little justification for retaining the outline from the fifth edition. A portion of the discussion of water bacteriology is based upon the 1933 edition of *Standard Methods for Water Analysis*.

F. E. NELSON

BACTERIOLOGY

345. **Inhibition of lactic acid bacteria by analogs of pantothenic acid.** WILLIAM DRELL AND M. S. DUNN. Univ. of California, Los Angeles. J. Am. Chem. Soc., 70, 6: 2057. 1948.

The growth of 23 strains of lactic acid bacteria, which require pantothenic acid, is inhibited by ω -methylpantothenic acid. The taurine analog showed less inhibitory action than the β -alanine derivative, but more than the derivative containing L-leucine. With four species of lactobacilli it was

shown that the inhibitory action of ω -methylpantothenic acid is reversed competitively by pantothenic acid over a wide range of concentrations.

H. J. PEPPLER

CHEESE

346. The manufacture of good quality cheese from pasteurized milk.

H. L. WILSON, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 44, 1: 28-31. July, 1948.

It will not be too long before practically all Cheddar cheese produced in this country will be made from pasteurized milk. A cheesemaker has complete control of the making process when he has a good quality milk that is properly pasteurized.

The intake room must be properly supervised, every can of milk must be inspected and only milk that can be made into good quality cheese accepted. The milk should be heated just high enough and held just long enough to show a phosphatase-negative test. A standardized procedure such as is suggested in detail should be followed.

To make further progress, the cheese industry should have a program including: (a) properly constructed buildings, with up-to-date sanitary equipment kept in such condition that the milk and curd will not become contaminated; (b) a good quality of clean milk whether it is pasteurized or not; (c) a good active uniform starter; (d) approved uniform methods of manufacture; (e) curing conditions that will permit the continued activity of essential bacteria which develop the characteristic flavor of Cheddar cheese.

F. W. BENNETT

CHEMISTRY

347. Recent developments in the vitamin A field. IAN HELLBRON. J. Chem. Soc., 1948: 386-393. March, 1948.

This material is the text of a lecture reviewing the chemistry of vitamin A, particularly from the standpoint of syntheses of the various compounds important in elucidation of structure and function.

F. E. NELSON

348. Structural relationships in the natural unsaturated higher fatty acids. T. P. HILDITCH J. Chem. Soc., 1948: 243-252. Feb., 1948.

This is the text of a lecture reviewing the structure and occurrence of these acids, particularly with respect to aquatic life and seeds.

F. E. NELSON

CONCENTRATED AND DRY MILK; BY-PRODUCTS

349. Dry milkfat as a form of storage fat. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 43, 5: 39, 46. May, 1948.

Various methods of preparation of dry milkfat have appeared in the literature. A practical method of production is described in the U. S. Patent 2,406,819 issued to Arthur W. Farrall and assigned to the Creamery Package Manufacturing Company.

Dry milkfat is produced from fresh cream. The principle involved is removal of water and serum solids from the cream by centrifugation. The cream is heated to pasteurizing temperature, preferably 170 to 190°, and separated into 80% fat. The plastic cream then is run through an homogenizer and into a continuous-type settling tank. The resulting product is approximately 98% fat. It is decanted and passed through an additional centrifuge to produce a dehydrated milkfat.

Standards of the U. S. Quartermaster Corps are: milkfat, not less than 99.8%; moisture, not more than 0.1%; copper, not more than 0.25 p.p.m.; peroxide value, zero; free fatty acids, not more than 0.5. Such dry milkfat can be stored at 40° F. for 6 months with little analytical or organoleptic change. At 0° it will remain in excellent condition for an indefinite period of time. Dry milkfat may be used any place that butter can be used.

F. W. BENNETT

FEEDS AND FEEDING

350. Preliminary observations on using a synthetic milk for raising pigs from birth. L. K. BUSTAD, W. E. HAMS, AND T. J. CUNHA, State College of Washington, Pullman. Arch. Biochem., 17: 249. 1948.

An attempt was made to raise pigs removed from the mother at birth and placed on a synthetic milk containing all known vitamins, crude casein, a liver preparation, brewer's yeast, lactose, and colostrum substitutes. Pigs fed no colostrum substitutes (blood serum and plasma) died shortly after birth. Severe diarrhea occurred in all pigs; it could not be controlled with penicillin, sulfathalidine, sulfamethazine and Kaopectate. The obviously inadequate diet failed to keep pigs alive more than 22 days.

H. J. PEPLER

FOOD VALUE OF DAIRY PRODUCTS

351. Sugar combined with milk in ice cream no tooth decay threat. ANONYMOUS. Ice Cream Rev. 31, 11: 160. June, 1948.

Sugar, when combined with milk or dry skim milk and butter, is not a

contributing cause to dental caries, according to results of a study recently conducted at the University of Wisconsin.

In experiments with cotton rats, milk was found to protect the teeth of rats against dental caries and afforded a certain degree of protection, even when the rats were fed a solid dry ration, which characteristically produced a great many carious lesions. When dry whole milk, dry skim-milk plus butter or casein-sucrose experimental diet was reconstituted with water and fed, a great reduction in dental caries was noted. It was concluded that a large part of the protective effect of milk is due to its fluidity. Other observations indicated particle size to be important in preventing tooth decay, since rations made up of very coarse sugar caused less dental decay than did rations of finely powdered sugar. W. J. CAULFIELD

ICE CREAM

- 352. Concentrated and dry ice cream mixes.** R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 43, 2: 110-11. Feb., 1948.

During 1945 approximately 100,000,000 lb. of dry and concentrated ice cream mixes were shipped to the armed forces. Tests indicated that the maximum length of time they would stand up at 100° F. was 4 months. The use of ice cream mix by the housewife and small ice cream manufacturers still is increasing.

Ice cream mixes have an excellent future, provided (a) high quality dry or concentrated mix is maintained by the manufacturer; (b) the manufacturer and user rotates his product and does not extent periods of storage excessively; (c) unnecessary regulations are removed from present legislation and new workable regulations are set up; (d) a realistic attitude toward the economy of exportation is maintained. F. W. BENNETT

- 353. New flavors for ice cream.** F. W. BENNETT, University of Georgia, Athens. Southern Dairy Products J., 41, 2: 55. Feb., 1947.

Two new flavors for ice cream are described, muscadine and peanut butter. The muscadines are prepared by washing and removing foreign material which may be present. The grapes then are pressed to remove most of the pulp and juice, leaving hulls and seeds. The hulls are heated to tenderize and passed through a colander to remove the seeds, after which they are combined with the pulp and juice. One lb. of sugar is added for each 4 lb. of fruit. Two or 3 quarts of the sweetened fruit is sufficient for 10 gallons of ice cream. The sweetened fruit also may be stored indefinitely at 0° F. or lower.

Some brands of peanut butter are superior to others for use in ice cream.

Two lbs. of the product is the proper amount to flavor 10 gallons of ice cream. If the butter is mixed with 1 lb. of dry sugar before adding to the freezer, its even distribution will be facilitated. Both flavors have been well received by local consumers.

F. W. BENNETT

354. **Factory-produced carry-out sundaes.** E. THOM, Assoc. Ed., *Ice Cream Review*. *Ice Cream Rev.*, 31, 11: 44, 45, 96. June, 1948.

The carry-out sundae, produced on a production line basis, is one means by which ice cream gallonage may be increased. At the Herron Ice Cream Company in Cleveland, a foursome package currently is being produced with considerable success. The package, approximately equivalent to 1 pint, consists of 4 ice cream rolls, approximately 2 inches in diameter, with a center core of fruit and pectin and a sprinkling of ground nuts, macaroons or almond bisque on the top.

A stainless steel extruder pipe is attached to the continuous freezer for producing the ice cream portion of the roll and the fruit is pumped to form the core through an inner stainless steel tube of any shape desired. Club, heart, diamond and spade combinations have proven to be the most popular core designs. The ice cream roll with its center core as it emerges from the 2 tubes, one inside the other, is fed onto stainless steel pans 3 ft. long. The pans move on a conveyor directly to the hardening room. Just before entering the hardening room, the pans pass beneath a device which sprinkles the top of the ice cream with ground macaroons or nuts, etc. After hardening, the ice cream roll is cut into any desired length. The individual portions then are placed into individual cellophane containers and packaged in units of 4 in cardboard boxes.

The package retailed for 35 cents in Cleveland at the time when ice cream was being sold for 30 cents a pint. This foursome package has proven extremely popular for sale directly into homes, and has enjoyed an excellent market among hotels, churches, lodges and other organizations holding large dinners. It has proven particularly helpful in keeping up sales during periods of low production.

The Esmond Dairy Company at Sandusky, Ohio, uses the same equipment to produce the sidewalk sundaes. This item consists of a roll of ice cream with a fruit core, but without nuts or macaroons being added. The ice cream roll of suitable length is wrapped with paper, which is easily peeled off, and is then inserted in a cone. This item was introduced during the depression period to combat double- and triple-dipped cones.

W. J. CAULFIELD

355. **The retail store of the future.** D. GHORMLEY. *Ice Cream Trade J.*, 44, 6: 56. June, 1948.

The ice cream store of the future will be located in a suburban business

district within a few blocks of a middle-class residential district and on a main boulevard. It will be found in a specially constructed building, 35 × 60 feet, which will have a large plate glass window, a 12-foot ceiling in the store area, a mezzanine over the storage area and wash rooms for customers. The fixtures will cost between \$7,000 and \$9,000 and will be made of colored plastics and stainless steel. All food preparations will be done in the kitchen and picked up by waitresses through pass-out windows. Dish washing will be done in a separate room. The store will seat 37 to 43 and will have 4 booths.

Standardization of food preparations, salesmanship and the daily operating technics will be utilized. The company president will be an experienced retailer and constantly on the alert for new ideas. Many side-line products will be handled by the store of the future. The store will be air conditioned, and have an "electric eye" door and a concealed nickelodian. There will be no home delivery or credit offered. W. H. MARTIN

356. **Borden's guides its dealers on retail selling prices.** ANONYMOUS. *Ice Cream Trade J.*, 44, 6: 46, 82-84. June, 1948.

The Borden Company letter announcing price increases on ice cream was accompanied by a brochure entitled, "How to Price Ice Cream." Prices recommended for hand-dipped half-pints, pints and quarts were 25, 45 and 85 cents, respectively. For sodas costing 6.94 cents, a 15-cent selling price was suggested. Dealers were cautioned to price correctly and to watch for consumer reaction to selling price as a guide to greater sales.

W. H. MARTIN

357. **Price trends.** ANONYMOUS. *Ice Cream Trade J.*, 44, 6: 44, 45, 93-98. June, 1948.

Higher cost of dairy products has caused ice cream manufacturers to increase the wholesale price of bulk ice cream 10 to 15 cents per gallon and the price of packaged ice cream 15 to 25 cents per gallon. In the East, bulk vanilla ice cream is priced at \$1.60 to \$1.80 per gallon, with quantity discounts. In Chicago the price reported was \$1.85 to \$1.87 per gallon on bulk vanilla. Prices in the South, Oklahoma, California and Kansas range from \$1.40 to \$1.50 per gallon and in Minneapolis \$1.20 to \$1.30 per gallon.

W. H. MARTIN

MILK

358. **Frozen storage of milk as a method of preservation.** F. J. DOAN, The Pennsylvania State College. *Milk Dealer*, 37, 8: 44, 102-112. May, 1948.

A discussion of frozen concentrated skimmilk, frozen fluid whole milk

and frozen concentrated whole milk which the author summarizes as follows: Bulk frozen concentrated skim milk is now being used, in a limited way, commercially for preserving milk solids-not-fat for later use, usually in ice cream. It has some advantages over other storage products and probably will be used more generally for this purpose. Another, frozen homogenized fluid milk, proved very satisfactory as a preserved substitute for fresh fluid milk for Army uses during the war where expense was not a limiting factor. It is unlikely, however, to become an important commercial article in the peace-time economy. The third, frozen concentrated whole milk can be employed for storing whole milk solids in bulk form and may at some future time appear on the retail market as one of the increasing number of frozen foods which can be held on hand in the home freezer or individual locker by the consumer. All three of these products are examples of an old method of frustrating nature's demolition corps, the microorganisms, but they represent new foods protected by such means.

C. J. BABCOCK

359. **Borden introduces oblong 2-quart glass bottle.** ANONYMOUS. *Milk Dealer*, 37, 8: 43. May, 1948.

Introduction of the space saving oblong two-quart glass milk bottle at food stores in Chicago and vicinity is announced by Borden's Chicago Milk Division. Thus, Chicago becomes the second city in the country to have this type of bottle, as it was introduced in New York some time ago.

The oblong bottle takes up 28% less space in a home or store refrigerator than the round half-gallon bottle and takes 33% less space than 2 single round quart bottles. The oblong container takes up 328 cubic inches of space as compared with 456 cubic inches for the round bottle. Another advantage of the oblong bottle is that it is easier to pour from. The oblong shape permits a firm grip on the sides.

C. J. BABCOCK

360. **Plant operation and efficiency.** L. C. THOMSEN, University of Wisconsin. *Milk Dealer*, 37, 8: 47, 48, 140-148. May, 1948.

Under operating costs, it is pointed out that on the basis of best available averages it appears that presently most fluid milk plants will require 36 cents to 45 cents of every sales dollar for operation, reserves for depreciation and a reasonable return on a justifiable investment. Of this amount, well over one-half is paid out directly for wages and salaries. Executives with officer titles receive less than one-half cent of the sales dollar. Under the heading "Labor Savings" filling is pointed out as the key operation, and if it is not properly timed as to output, efficiency is inclined to suffer.

The following proposals for sales economies are given: (a) increase size of retail and wholesale loads by use of lighter containers and cases; (b) use

public utility types of practices to restrict areas of operation; (c) expand store sales, which have been shown to cut sales costs to less than one-half of those for retail delivery; (d) establish numerous milk or dairy stores; (e) still further curtailing services to the consumer by, for example, delivery every third day; and (f) offer quantity discounts.

In discussing ways of reducing milk losses, the author shows that total "paper" losses in ideally operating milk plants selling fluid milk and cream amount to approximately 1.65%, of which slightly less than three-fourths can be assigned to the fluid milk part of the operations, and slightly over one-fourth can be charged to the fluid cream part of the operation.

H.T.S.T. systems, proper routing of milk, methods of reducing waste and care of equipment are also discussed. C. J. BABCOCK

361. **New products developments leading to greater milk sales.** K. G. WECKEL, University of Wisconsin, Madison. *Milk Dealer*, 37, 8: 50-56. May, 1948.

Preventing oxidized flavor, unsterilized evaporated milk, uniform equipment, extended delivery routes and electronic temperature recorders are discussed as potentialities that can improve and affect the sales and distribution of market milk. C. J. BABCOCK

362. **The manufacture of a high quality chocolate milk drink.** BENJAMIN P. FORBES, Benjamin P. Forbes Co., Cleveland, Ohio. *Southern Dairy Products J.*, 44, 1: 42-3. July, 1948.

Due to its greater miscibility, cocoa powder rather than chocolate is used in the preparation of chocolate milk drinks. The necessity for scrupulously observing the rules prescribed by the flavor manufacturer is obvious. In using reconstituted milks, the solids should be the same as in fresh milk.

Settling may be caused by: (a) insufficiently stabilized cocoa; (b) too much milk or insufficient butterfat content; (c) dilution of dairy drink by milk or water; (d) restriction of valves on pressure side of pumps, causing a homogenizing effect or excessive whipping by centrifugal pumps; (e) too acid milk (do not neutralize); (f) using soda-neutralized milk; (g) using frozen milk; (h) incorrect pasteurizing and cooling; (i) insufficient heat or too short holding time; (j) over-agitation during and after cooling; (k) powder not entirely incorporated; (l) not cooled to 40° or lower; (m) precooling below 140° in the vat.

Thickening may be caused by: (a) excessive pasteurizing temperature; (b) overstabilization; (c) use of lime or magnesium stabilizers; (d) improperly rinsed bottles; (e) milk which is too acid. F. W. BENNETT

- 363. Factors to consider in making high quality chocolate milk drinks.** W. C. THACKER. Southern Dairy Products J., 43, 2: 102-3, 116. Feb., 1948.

Chocolate milk is not consumed as a substitute for white milk. In 3 urban markets, the total per capita consumption of milk was 18% higher in the families that included chocolate milk.

The 6 desirable characteristics of chocolate milk or chocolate drink are: (a) High quality milk or part-skimmed milk. The minimum fat standard for whole milk should be met. (b) Mild chocolate flavor. The amount of flavor used ranges from 1.0 to 1.5% cocoa or 1.5 to 2.25% liquor chocolate. Cocoa is most used. Quality varies widely. Prepared syrups are convenient for small distributors, but the addition of the dry ingredients before pasteurization is less expensive. (c) Little or no sedimentation with a low to medium viscosity. Special finely-ground cocoas are on the market. Sodium alginate will prevent sedimentation at a low viscosity. Homogenization increases sedimentation. (d) Elimination of the ragged dark-colored cream layer. This can be prevented by pasteurization at 160 to 165° F. for 15 to 30 minutes and the addition of stabilizers. (e) Light to medium red-brown color. (f) Medium to high sweetness. Consumer demands differ from about 5 to 8% sugar. Standard composition and processing methods are important.

F. W. BENNETT

- 364. Production can be leveled.** H. F. SIMMONS, Michigan Milk Producers' Association. Milk Dealer, 37, 8: 92-100. May, 1948.

A discussion is given of the "Base and Surplus Plan" as a means of leveling the production of milk by farmers so that it more nearly conforms to fluid milk requirements.

C. J. BABCOCK

SANITATION AND CLEANSING

- 365. New sanitary practices and controls in the dairy industry.** M. G. PEDERSON, Price's Creameries, Inc., El Paso, Texas. Southern Dairy Products J., 42, 1: 34-5, 42-3. July, 1947.

Sediment may be received in the ice cream plant through milk and its products, sweetening agents, stabilizers and even the city water. In the plant, sediment may be introduced from solid flavoring materials, air in the plant, undissolved particles of cleaning and sterilizing agents, lubricants not held back by packing glands and cartons which have not been fully protected. Unsanitary practices of employees are another factor. Insects and rodents are important. Applying a 5% or stronger emulsion of DDT to the walls and ceiling every 2 or 3 weeks should control flies. Sodium fluoride, pyrethrum, rotenone and roach poisons, when regularly and prop-

erly used, are effective against cockroaches. Daily cleaning of the plant and the elimination of cracks and unused equipment which may harbor them are essential. Mouse-proof construction, trapping, poisoning and the elimination of rubbish are means of exterminating mice and rats. Antu, thallium sulfate, barium carbonate, zinc phosphide and strychnine should be used by an experienced and intelligent person. F. W. BENNETT

366. Cleaning and disinfecting milk and ice cream processing equipment.

M. A. BAILEY, The Diversey Corporation, Chicago, Ill. Southern Dairy Products J., 41, 4: 73, 78-81. Apr., 1947.

Clean-up operations consume over one-third of the labor in the dairy and therefore are worthy of serious consideration. In the 1920's, soap and washing soda were the two commonly used cleaning agents. Soap left a film of soap or scum on the equipment, did not possess the necessary cleaning power and was expensive to use. Action on the hands accompanied the use of washing soda, lime films were left and its cleaning performance was still far from efficient. Chemists set out to blend the various sodas with trisodium phosphate and metasilicate to obtain balanced cleaners.

In selecting the product to be used for general cleaning, the cleaner must remove contamination quickly and safely; no dirt or deposit must be left on the equipment; the cleaner must not damage the equipment; the cleaner must not be hard on the hands of the operator and must be liked by him; it must be economical to use judged by the results obtained.

There are six essential characteristics that a good cleaning compound should exhibit: water softening to eliminate a mineral build-up on the equipment; wetting action to penetrate and wet the film to be removed; suspending action to prevent the settling out of soil to re-adhere to the equipment; emulsifying ability to keep fats suspended and allow them to be rinsed off easily; rinsing ability so that the cleaner itself may be easily removed; bactericidal action in special operation such as bottle washing.

Milkstone may be removed safely and prevented by the successive use of specially prepared acid and alkaline cleaners in short-time pasteurizers, vacuum pans and evaporators.

Training of the workers in the care and cleaning of equipment is of the utmost importance. The importance of their work must be impressed upon them for best results. Trained representatives of several chemical companies will assist gladly in an educational plan for plant employees.

F. W. BENNETT

MISCELLANEOUS

367. Refrigeration for dairy plants. A. A. GEIGER, York Corporation, Atlanta, Ga. Southern Dairy Products J., 39, 5: 82, 88-9. May, 1946.

The refrigeration for dairy plants should be designed to handle easily

the maximum load, require no hand regulation of control valves for minimum loads and have sufficient flexibility. All efforts should be made to make the plant free of shut-down possibilities. There are 3 types of refrigerating mediums for cooling milk products: brine, direct expansion, or sweet water. For the last 10 years, milk machinery manufacturers of vertical vats with spray jackets have tended to recommend cooling by sweet water rather than brine. If an open type sweet water tank is used, an ice field is built up during the night to serve as a storage of refrigeration. As the ice melts during milk cooling, the temperature of the sweet water reaching the milk cooler may increase 20°. For this reason, this system cannot be recommended for milk plant use. The shell and tube water cooler to deliver 34° F. water is subject to freezing up. Instantaneous water coolers with controls to deliver water at uniform optimum temperature are available. Brine for cooling makes it possible to use a smaller milk cooler because of a lower brine temperature. When brine is used, only one ammonia control is necessary. The compressor in a brine system can be operated at 100% capacity. The wide range of temperature possible in the brine enables the plant to have a low enough temperature at all times.

Corrosion should not be a problem if the brine is not allowed to become acid. Brine may be used in a plate cooler if the tightening head is equipped with a compensating spring and a proper brine control is used. The direct expansion system is not advisable for small plants because of (a) excessive cost on small volumes, (b) complicated controls and (c) short-cycling of compressors. This system adapts itself advantageously in larger plants, but for buttermilk and cream cooling, the sweet water system is used.

A two-compressor installation is recommended rather than a single compressor for flexibility. Too few operators of milk plants devote enough attention to their refrigeration needs.

F. W. BENNETT

368. How employer-employee relationships reflect good management.

J. W. Post, Armour and Co., Chicago, Ill. *Ice Cream Rev.*, 31, 11: 76, 78, 80, 82, 84. June, 1948.

The losses or gains as a result of customer satisfaction or dissatisfaction depend upon the kind of personnel developed by the company. Management must assume the responsibility for seeing that the right kind of personnel is developed, as only a few good employees are self-made. Fourteen groups of desirable traits and habits which should be developed in employees are enumerated.

Better employer-employee relationship may be developed in many ways. Some of the methods currently used by well-managed businesses include the following: employee publications to improve the line of communications between employees and the company; job evaluation as it applies to wage

rates to prevent inequities; polling of employees for opinions, ideas and suggestions for improvement; regular training and educational procedure which is oral, visual and written; aptitude and psychological testing that help in employee selection and in job assignments; periodic merit rating by procedures which meet the need for individual appraisal; provision for recreational and health protection facilities; and posting of employment policies.

Another factor to be considered is that of federal and state legislation as it affects employer-employee relationships. Plans must be formulated to meet such things as sick-benefit plans, life, health and accident plans, profit-sharing or merit-rating plans. The position of management with respect to labor relations is entirely different from that of a few years ago. Business now is confronted with inflation, full employment and much new legislation which affects employer-employee relations.

Management must devote more time to the personnel problem. What your employees think and express determines in a large measure the public relations of the company concerned. How well the employer-employee relationship problem is solved will determine in no small measure the success of a given business under present-day conditions. W. J. CAULFIELD

369. Economic aspects of open and closed cream markets. LELAND SPENCER, Cornell University. Milk Dealer, 37, 8: 86-88. May, 1948.

Following a listing of the consequences of maintaining strict requirements as to conditions under which cream is produced and handled and also limiting the area of inspection, it is concluded that: (a) the "closed" market policy for cream does not result in higher cost to consumers for fluid milk; (b) the extra income for producers is obtained through higher prices for cream than would prevail in an "open" market; (c) the extra cost of cream is shared by consumers and distributors of fluid cream and sour cream and by manufacturers of ice cream.

Another point worthy of emphasis is that the milk produced for use as cream in a "closed" market is a reserve supply that can be drawn upon for use as fluid milk whenever the milk supply from inspected sources is insufficient for all demands.

The advantages and disadvantages of requiring cream to be produced and handled under strict sanitary requirements within a limited production area are difficult to evaluate. Considerations of public health and aesthetics as well as economics are involved. There is need for a thorough and careful determination and appraisal of pertinent facts as a basis for intelligent action.

C. J. BABCOCK

370. Trend toward conversion to oil as fuel continues. ANONYMOUS.
Milk Dealer, 37, 8: 40. May, 1948.

A survey made by the Olsen Publishing Co. of milk dealers in New England and North Central states shows that the trend among milk dealers toward converting plants from coal to oil is continuing.

Out of 434 replies received to questionnaires, 54.5% reported they are now using oil as fuel, 43.4% are using coal, and 3.1% are using gas. Among these same dealers, however, 14.1% indicated they are planning to make a change in the type of fuel used, and of these, 71.5% expect to change from coal to oil and another 10.7% from coal to gas.

The determining factor mentioned most often by respondents to the questionnaire was that oil or gas was cleaner than coal. Second most popular determining factor was that oil or gas saves labor; third was convenience; fourth was price; and fifth was efficiency. C. J. BABCOCK

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

- 371. Proteins and amino acids in nutrition.** M. SAHYUN, editor. 566 pp. \$7.50. Reinhold Publishing Corporation, New York, N. Y., 1948.

Eighteen eminent authorities associated with various fields of nutrition have contributed to this volume, which contains a wealth of information relating to the nutritional role of proteins and amino acids. The first chapter is devoted largely to a historical review of research related to the subject. A number of subsequent chapters consider the general aspects of the role of protein in nutrition, whereas others deal with specialized subjects such as hormones, plasma proteins, toxins and filterable viruses. Although the subject matter is treated primarily from the standpoint of human nutrition, one chapter is devoted to the amino acid requirements of the avian species, and frequent reference is made to other species, especially the bovine. A comprehensive list of literature references is presented at the end of each chapter and an extensive index covers the subject matter of the entire book. The appendix contains two tables which indicate the nutritive value of a large variety of human foods.

This book should be of interest to all who are concerned with the fields of either human or animal nutrition.

N. L. Jacobson

BREEDING

- 372. Hereditary epithelial defects in ayrshire cattle.** F. B. HUTT AND J. N. FROST, Cornell University. *J. Heredity*, **39**: 131-137. 1948.

An epithelial defect, less severe but otherwise similar to that found in Holstein-Friesian cattle by Hadley and Cole, was found in 4 registered Ayrshire calves. Raw areas were found in knees and distal extremities of all four limbs just above the hoofs and, to a lesser degree, on the muzzle. Mucosa was lacking under the tongue in one calf examined. Another calf had lesions on the inner surface of the ears. Three calves were vealed at 2-3 weeks. One calf that was protected against bacterial invasion by sulphathaladine prophylaxis was destroyed at 3.5 months. Breed difference was suggested as being due either to genotype in which the mutant operates or, in the case of Jerseys, to a different gene. The defect apparently was caused by a single autosomal recessive gene carried by the sires, Whitpain Skytop and Mansfield May's Augie.

L. O. Gilmore

CHEMISTRY

373. The glyceride composition of milk fat. C. P. ANANTAKRISHNAN, V. R. BHALERAO, AND T. M. PAUL, Indian Dairy Research Institute, Bangalore, India. Arch. Biochem., 18, 1: 35-40. July, 1948.

Sindhi cows were fed a basal ration supplemented with cottonseed, sesame and hydrogenated cocoanut and groundnut oils at the rate of 1.5 lb. per head per day. In comparison with the butter fat of the control group, reduced Reichert values and increased iodine values, butyrefractometer readings and saponification values were observed. With the exception of the group fed hydrogenated cocoanut oil, a reduction in the total amount of acids up to C_{14} occurred. Supplements of cottonseed, sesame and hydrogenated groundnut oils led to an increase in the oleoglycerides of the milk fat. Rations with a high percentage of linoleic acid failed to show increased amounts of this constituent in the milk fat.

H. J. Peppler

374. A browning reaction involving copper-proteins. J. B. THOMPSON, R. B. KOCHER, AND H. W. FRITZSCHE, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill. Arch. Biochem., 18, 1: 41-50. July, 1948.

A browning reaction produced by copper-protein complexes is described. The mechanism is different from known types of enzymatic browning. Reactions of the copper-proteins of casein, gelatin and albumin with compounds containing an ethylene group within a ring structure are discussed. Tryptophane, cystine and an acid hydrolyzate of casein did not replace casein in the browning reactions, thus indicating that copper was bound through forces existent in polypeptides rather than amino acids.

H. J. Peppler

CONCENTRATED AND DRY MILKS; BY-PRODUCTS

375. Process for manufacture of milk sugar. D. D. PEBBLES AND T. V. MARQUIS. (assigned to Western Condensing Co.) U. S. Patent 2,439,612. 8 claims. April 13, 1948. Official Gaz. U. S. Pat. Office, 609 (2): 400. 1948.

Whey first is condensed after receiving sufficient heat by direct steam to coagulate the protein. The condensed product, supersaturated to lactose and free of lactose crystals then is heated by direct steam, cooled by evaporation and seeded with lactose. After mass crystallization, the lactose is removed by centrifugal force, washed and dried.

R. Whitaker

DISEASES

376. Some observations on postparturient cows in four separate herds as related to expulsion of their fetal membranes. W. L. BOYD AND A. F. SELLERS. Cornell Vet., 38, 3: 263-266. July, 1948.

Data from 1 Bang's-positive and 3 Bang's-negative herds are presented in

relation to expulsion of fetal membranes. In a series of 542 parturitions, rates of placental retention appeared high in cases of abortion and stillbirth regardless of Bang's status, but the rate of retention was approximately 3 times higher in the Bang's-positive herd than in the Bang's-negative herds, in the viable, single-calf group. It also was observed that retention rates were higher in viable, single-calf gestations terminating outside of the normal range of 274-291 days than in those terminating within normal range.

T. M. Ludwick

FEEDS AND FEEDING

377. All-year pasturing with and without concentrates for dairy cows.
B. P. HAZLEWOOD, Univ. of Tennessee, Knoxville. Tenn. Agr. Expt. Sta.
Bull. 207. 1948.

Two groups of 16 Jerseys were used in experiment. One group received no grain, the other group received grain at the rate of 1 lb. to each 3 lb. milk produced. Each group received all-year pasture, alfalfa hay and silage. Individual weights, production and feed-consumption records were kept. The experiment ran over a period required for the cows in each group to complete 2 successive lactations. Production of cows in the no-grain group was 76% of that of grain-fed group. Each group followed the same seasonal trend of production, but the grain-fed cows were the more persistent.

W. Dudney

378. Mineral metabolism studies in dairy cattle. IV. Effects of mineral supplementation of the prepartal diet upon the composition of the blood of cows and their calves at parturition. J. T. REID, G. M. WARD, AND R. L. SALISBURY. N. J. Agr. Expt. Sta., Sussex. J. Nutrition, 36, 1: 75-89. July 10, 1948.

Four groups of cows were fed a basal concentrate ration containing 1% calcium plus hay, silage and pasture in season during at least the last 2 months of gestation. Group I received the basal concentrate, group II the basal concentrate plus 2.5% calcium carbonate, group III basal concentrate plus 3% calcite flour and group IV basal concentrate plus 3% mica. The supplement for groups III and IV contained various trace elements. Blood or plasma analyses were made for 49 dams and 50 calves just following parturition. Only 7 of the calves received colostrum. These supplements had no effect upon levels of calcium, phosphorus and several other blood constituents. However, both cows and calves of group III had lower levels of reduced and total glutathione than other groups. Calves blood contained greater concentrations of reduced and total glutathione, calcium, inorganic phosphorus, acid and alkaline phosphatase and ascorbic acid and greater numbers of erythrocytes than their dams. The dams had greater corpuscular hemoglobin content and corpuscular volume and higher levels of total proteins, globulin and albumin. Sex had no effect on constituents of blood of calves.

R. K. Waugh

HERD MANAGEMENT

379. **Efficient mechanical milking.** W. G. WHITTLESTON. *Australian J. Dairy Technol.*, **3**, 2: 45-72. 1948.

This article reviews 3 aspects of the problem—the influence of various factors on the process of getting milk from the cow, the construction of the milking machine and the installation, care and servicing of the machine. The discussion is of such length that detailed abstracting is not practical. The author approaches his subject in a thoroughly critical manner; he is an advocate of simplification of the machine and of its use.

F. E. Nelson

ICE CREAM

380. **Foreign fats in ice cream.** ANONYMOUS. *Ice Cream Trade J.*, **44**, 7: 34. July, 1948.

The Texas State Board of Health has ruled that all ice cream shall contain 8% butterfat to which may be added 4% vegetable fat. Prior to this ruling, all state laws and regulations have clearly specified that the fat content of ice cream must come from dairy products except for the fats present in such items as chocolate, but even in such cases, only a reasonable tolerance has been allowed. The International Association of Ice Cream Manufacturers and the Texas Dairy Products Institute are trying to get the ruling changed.

W. H. Martin

381. **Ice cream package for the elite.** ANONYMOUS. *Ice Cream Trade J.*, **44**, 7: 32. July, 1948.

A new product in a transparent plastic container, representing a radical departure in ice cream package merchandising, has been introduced in the New York market by the Rosemarie de Paris, one of the country's most distinctive chocolate and confectionary firms. The ice cream, a French type containing 18% butterfat and low in overrun, is made in 5 basic flavors—vanilla, chocolate, coffee, strawberry and pistachio. Pints retail for 85 cents and quarts for \$1.60.

The product is packaged in a new transparent acetate tub-shaped container, measuring 3 inches in diameter at the bottom, $3\frac{1}{2}$ inches at the top and about 4 inches high. The lid is a disk-shaped paperboard. The famous Rosemarie de Paris coach, the company's trade mark, is reproduced on the package but does not hide the contents which are plainly visible.

W. H. Martin

382. **Carton for ice cream and the like.** S. H. BERCH. U. S. Patent 2,443,530. 4 claims. U. S. Patent 2,443,531. 2 claims. June 15, 1948. *Official Gaz. U. S. Pat. Office*, **611** (3): 721. 1948.

A container for ice cream and other frozen foods is described which is cut from 1 piece of material and scored in such a manner that it may be folded to produce a carton held in shape by interlocking flaps and requiring no adhesive.

R. Whitaker

- 383. Brine tank.** J. H. REAGIN. U. S. Patent 2,442,146. 3 claims. May 25, 1948. Official Gaz. U. S. Pat. Office, **610** (4) : 942. 1948.

The distinguishing feature of this brine tank for making frozen confections is its shape. The tank is round, the product freezing in the molds during one revolution around the tank. Cold brine is supplied continuously from a supply tank equipped with refrigeration coils.

R. Whitaker

- 384. The retail store.** D. GHORMLEY. Ice Cream Trade J., **44**, 7: 40. July, 1948.

There seems to be good economic justification for the specialized ice cream store. These stores can give the best of service; the stores are built and equipped and the personnel are trained to eliminate problems commonly faced by customers in search of ice cream. The stores are conveniently located, they sell for cash and they can sell for less and make a profit. There are some disadvantages to this type of merchandising. The season is short, the hours are long, the product is perishable and competition plentiful. The ice cream merchant must weigh these advantages and disadvantages and then determine his course of action.

W. H. Martin

MILK

- 385. Heat exchanger.** F. P. HANRAHAN. (Assigned to people of the U. S.) U. S. Patent 2,445,115. 2 claims. July 13, 1948. Official Gaz. U. S. Pat. Office, **612** (2) : 422. 1948.

In a device for heating milk and other fluids and having such desirable features as sanitary construction, rapid continuous heating and compact size, the milk is caused to flow at high velocity in a spiral channel between two heating surfaces. Only two relatively small replaceable gaskets are employed.

R. Whitaker

- 386. Milk bottle closure.** F. M. ALEXANDER. U. S. Patent 2,442,745. 1 claim. June 8, 1948. Official Gaz. U. S. Pat. Office, **611** (2) : 410. 1948.

A closure for glass milk bottles is described consisting of a hinged disk which may be raised or lowered by pressing on a wire handle. The device is attached to the bottle neck by means of a spring jaw.

R. Whitaker

- 387. Cover and filter for milker pails.** T. J. PFETCHER. U. S. Patent 2,445,122. 1 claim. July 13, 1948. Official Gaz. U. S. Pat. Office, **612** (2) : 444. 1948.

A combination cover and filter for milk pails is described. R. Whitaker

SANITATION AND CLEANSING

- 388. Sterilizing arrangement for milk tubes.** J. L. FAIR. U. S. Patent 2,441,878. 3 claims. May 18, 1948. Official Gaz. U. S. Pat. Office, **610** (3) : 710. 1948.

A device is described for maintaining the rubber teat cups of milking machines full of a sterilizing solution when not in use. R. Whitaker

389. Apparatus for washing milking machines. R. C. HERMAN. U. S. Patent 2,442,926. 1 claim. June 8, 1948. Official Gaz. U. S. Pat. Office, 611 (2) : 454. 1948.

A tank containing water and under air pressure is situated under a basin for cleaning milking machine parts. From the tank, air and water lines, equipped with valves, feed a nozzle over the basin. R. Whitaker

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

- 390. The artificial insemination of dairy cattle. A handbook and laboratory manual.** H. A. HERMAN AND F. W. MADDEN. 95 pp. Lucas Bros., Columbia, Mo. 1947.

This manual is designed especially for use in teaching laboratory work in courses dealing with the artificial insemination of dairy cattle. It also should be of interest and value to managers, technicians and field workers in artificial breeding associations, as well as others interested in the technics employed.

Twelve of the 20 exercises deal with the collection, examination, dilution, storage and transportation of bull semen and insemination of the cow. Particular emphasis is placed on laboratory methods for evaluating the quality of semen. Brief illustrated discussions of the anatomy and physiology of the reproductive organs of the bull and cow are presented, along with exercises covering pregnancy determination and reproductive troubles of dairy cows. In addition to reviewing some of the factors involved in the selection, management and care of sires, there are exercises pertaining to the organization of artificial breeding associations and record keeping.

The questions and references found at the end of each exercise should be of special aid to the student in comprehending and enlarging upon the principal points presented. For teaching at the college level, the authors suggest that this handbook be supplemented liberally with more detailed lecture material.

J. O. Almquist

- 391. Standard methods for the examination of dairy products.** 9th Edn. \$4.00. American Public Health Association, 1790 Broadway, New York 19, N. Y. 1948.

This long-awaited new edition of the standard publication in this field constitutes a distinct departure in the presentation of the material covered. Because of the expanded coverage, cross references have become essential to avoid much undesirable duplication. The separation of discussion of application from the actual directions for the test in question should simplify use of the book by the various interested groups. Drs. Robertson and Black are to be commended for their editorial work, for they have integrated the material on the various tests in a manner which makes a very definite contribution to the usefulness of the publication.

Only a few of the specific changes will be enumerated. Incubation of plates

for plate counts now is specified at either 32 or 35° C. (No indications of tolerances allowable are given.) The 1-hour and triple-reading procedures for the resazurin test are recognized. Hourly inversion of both methylene blue and resazurin reduction tests becomes the standard procedure. The section on sediment testing has been enlarged considerably. Laboratory procedures for the phosphatase test are recognized in the main body of the publication, and field tests are incorporated in the chapter on "Screening Tests". In this chapter also are included many tests or modifications of tests which will serve for routine control but which are considered unsuitable for official or regulatory action. In addition to methods for assay for vitamin D, methods for thiamine, riboflavin and niacin are given, being quoted from the twelfth revision of the *United States Pharmacopoeia* and the second bound supplement therof.

This ninth edition of *Standard Methods* is a volume which should be consulted repeatedly in every dairy control laboratory in the country and should be read, at least in part, by all who have occasion to use or interpret the results of laboratory control of dairy products in manufacturing or merchandising activities.

F. E. Nelson

BACTERIOLOGY

392. Newly proposed staining formulas for the direct microscopic examination of milk. B. S. LEVINE AND L. A. BLACK, U. S. Public Health Service, Cincinnati, Ohio. *Am. J. Pub. Health*, **38**, 9: 1210-1218. Sept., 1948.

A two-dip staining procedure was adopted for study using 0.6% methylene blue in 95% ethyl alcohol to stain the prepared smears after defatting. Also a single dip stain, free from water and acid was prepared as follows: 0.6 g. certified methylene blue powder, 50 ml. of 95% ethyl alcohol and 50 ml. of technical grade tetrachlorethane. A comparison of the carbolated methylene blue stain, Newman-Lampert stain and the water- and acid-free stain proposed by the authors showed that bacteria and leucocyte counts of raw milk obtained by the carbolated methylene blue and the Newman-Lampert stain agreed closely. However, in the smears stained by the authors' procedure, the bacteria count averaged 94% higher and the leucocyte count averaged 9% below either of the other two methods used. The use of a polychrome methylene blue stain resulted in counts of both bacteria and leucocytes higher than when the carbolated methylene blue stain was used, but lower than those obtained with the acid- and water-free stain. No polychrome effects were noticed in the raw milk smears. The data obtained indicate that the water- and acid-free stain will yield maximal counts.

D. D. Deane

393. The bacteriological examination of ice cream. Part 2. JOYCE CRANFIELD, Edinburgh University. *Dairy Inds.*, **8**, 8: 800-804. Aug., 1948.

One hundred samples of ice cream were tested by a modified methylene blue test with a pre-incubation period of 17 hours at atmospheric shade temperature below 20° C. Ninety per cent of samples with plate counts of more than 200,000

organisms per ml. and *E. coli* present in 0.01 ml. were graded as 3 or 4 by the methylene blue reduction test. One hundred per cent of samples with plate counts of less than 200,000 organisms per ml. were graded 1 or 2 by the methylene blue test.

Results are given on 20 samples of ice cream to show the change in the total count and *E. coli* content after (a) refrigeration overnight and (b) standing at atmospheric temperature overnight. Overnight refrigeration tends to bring about a slight increase in plate count but a decrease in the coliform content. In most cases, the correlation between the plate count and coliform content and the grading of the samples according to the methylene blue test was unchanged by overnight refrigeration. The plate count always increased and the coliform content frequently increased when samples were held at room temperatures overnight. Little correlation was found between the results of the tests carried out on the second day and the grading of the samples according to the methylene blue test on samples held overnight at room temperature. G. H. Watrous

394. The resazurin test—a review of the literature from 1940–1948. D. W. WATSON. Dairy Inds., 8, 8: 751–762. Aug., 1948.

A fairly complete collection of the most important papers from 1940 to 1948 is presented under the headings: (a) Synthesis and standardization of the dye; (b) Technic; (c) Fundamental work; (d) Mastitis and abnormal milk; and (e) Modifications, practical applications, and comparisons with other tests.

G. H. Watrous

395. Esters of vanillic acid as spore-controlling agents. F. R. EVANS AND H. R. CURRAN. Bureau of Dairy Industry, U.S.D.A., Washington, D.C. Food Research, 13, 1: 66. Jan.–Feb., 1948.

The spore-controlling action of 4 vanillic acid esters in milk at 0.10 and 0.15% concentrations was studied over periods of 3 to 4 months against cultures comprising 20 mesophilic aerobes, 10 thermophilic aerobes, and four mesophilic anaerobes, temperature of storage being 20, 37 or 52° C.

Isobutyl vanillate was the most efficient preservative. In 0.15% concentration, all samples remained unchanged over the storage period. N-butyl vanillate was slightly less effective, particularly against anaerobes. Ethyl vanillate delayed but seldom prevented growth. Sodium benzoate in 0.15% concentration in milk was relatively ineffective as a spore-inhibiting agent but had little effect on flavor. However, a like amount of the vanillates produced a distinctly objectionable flavor. The action of the reagents was judged to be primarily sporistatic.

F. J. Doan

396. A new and rapid method for the preparation and standardization of Brucella Ring Test antigen. R. M. WOOD, Dept. of Bact., Johns Hopkins School of Medicine, Baltimore, Md. Am. J. Pub. Health, 38, 9: 1225–1227. Sept., 1948.

The author proposes a new and more rapid method of preparing the antigen

used in the Ring test for *Brucella* agglutinins in milk. A smooth strain of *Brucella abortus* is grown in broth for 24-48 hours to obtain massive growth, harvested, treated with phenol and held at 60° C. for 1 hour, then chilled, centrifuged and the supernatant discarded. After resuspension in distilled water, the cells are filtered through glass wool, washed twice more and then resuspended in 2 volumes of distilled water. The suspended cells are then stained with hematoxylin, centrifuged down and washed with distilled water until the supernatant is colorless. After resuspending in 0.85% saline containing 0.5% phenol, the stained antigen is standardized and checked for sensitivity. Substitution of the stained *Brucella* antigen for the unstained antigen normally used in either the slow tube or rapid plate method of serum agglutination results in an endpoint that is seen more easily. The author found the Ring test method, using the stained antigen as described, was fully as sensitive and had fewer sources of error than the whey titration for detecting *Brucella* agglutinins in milk. D. D. Deane

397. The bacterial activity of "racemized casein", caseose, and the four diastereoisomeric leucylleucines. S. W. FOX, Y. KOBAYASHI, S. MELVIN, AND F. N. MINARD, Chem. Lab., Ia. State College, Ames. J. Am. Chem. Soc. 70, 7: 2404-2406. 1948.

Experiments with racemized casein and caseose indicate that a polypeptide preparation containing a larger proportion of D-amino acid residues than occurs in tyrocidine is without appreciable antibacterial activity when tested against *Escherichia coli* or *Lactobacillus arabinosus* 17-5. H. J. Peppler

398. Pasteurization of liquid-egg products. IV. Destruction of coliforms. A. R. WINTER AND G. F. STEWART, Ia. Agr. Expt. Sta., Ames, AND MARIAN WILKIN, Ohio State Research Foundation, Columbus. Food Research, 13, 1: 11. Jan.-Feb., 1948.

Coliform bacteria were found in all (134) samples of commercial liquid, frozen and defrosted whole egg. Those found in unfrozen, liquid whole egg samples were destroyed more easily by pasteurization than those found in defrosted liquid whole egg samples. Pasteurization of unfrozen and defrosted liquid whole egg samples at 146° F. for 0.5 minute, 144° F. for 1.0 minute, 142° F. for 1.5 minutes and 140° F. for 2.5 minutes totally destroyed the coliform bacteria in all but one of the samples. F. J. Doan

CHEESE

399. Composition control of cheddar cheese. H. L. WILSON, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 44, 2: 30-42. Aug., 1948.

The following conditions tend to increase the moisture content and the opposite conditions tend to decrease it: less starter, more rennet, cutting curd as firm as possible, lower cooking temperature within a range of 98-102° F., less agitation, pushing back curd sooner before draining, packing curd deeper, cutting into wider

slabs, piling sooner, faster and higher, lower temperature during cheddaring, shorter cheddaring time, milling into larger pieces, shorter periods of forking after milling and salting, shorter holding period after salting and the use of larger size hoops.

Lower moisture content of the curd and the addition of the salt more slowly and in more applications tend to increase the salt content. The uniformity of salt content is increased by uniform distribution of salt, uniform depth of curd in vat, forking curd from ends and sides into the center of vat, and ditching the curd to allow the whey to run off. Piling the curd and allowing it to stand as long as possible, but still be able to free it of lumps, will tend to eliminate mechanical holes. Alternate piling and forking must be repeated until the salt is thoroughly dissolved.

F. W. Bennett

- 400. Brown discoloration in malted process cheese.** I. HLYNKA AND E. G. HOOD. Division of Chemistry and Division of Bacteriology and Dairy Research, Dept. of Agriculture, Ottawa, Canada. Food Research, 13, 3: 213. May-June, 1948.

Experiments with malted cheese (a blend of process cheese with malt syrup) revealed that the browning defect to which this cheese is subject is caused by an interaction of amino acids and proteins of the cheese with aldose sugars in the malt and results in an accompanying increase in titratable acidity and lowering of pH. It was concluded that malted cheese can be protected from this color defect by additions of sulfites or sulfur dioxide, the use of which is regulated in foods by the various departments of health and/or foods chemistry.

F. J. Doan

- 401. Cottage cheese.** G. C. NORTH, AND L. LITTLE. (Assigned to Beatrice Creamery Co.). U. S. Patent 2,446,550. 3 claims. Aug. 10, 1948. Official Gaz. U. S. Pat. Office, 613 (2), 364. 1948.

Cottage cheese is prepared by cutting enzyme coagulated curd at a whey acidity of between 0.25 and 0.45%, cooking to obtain a rubbery tough body, freezing the curd and then thawing to produce a firm bodied and smooth textured product.

R. Whitaker

CHEMISTRY

- 402. Vitamin A content of Cuban Cow's milk and of liver oils of Cuban sea sharks.** J. J. ANGULO, R. F. COWLEY, ALBERTO MAIRERO, AND CESAR FUENTES, School of Medicine, Havana University and Laboratory of Compania de Pesca del Valle, S. A., Havana, Cuba. Food Research, 13, 1: 1. Jan.-Feb., 1948.

Fifteen samples of cow's milk obtained from Havana cafeterias during the late summer and autumn indicated that boiled milk contained the greatest quantity of vitamin A and carotene, followed in order by Grade A milk (raw or pasteurized). The higher levels encountered in the boiled milk were attributed to the loss of water as a result of boiling. The levels of vitamin A and carotene

in Cuban Grade A milk were higher than values in the literature for Guernsey milk and considerably higher than for Holstein, Brown Swiss and Jersey milk.

F. J. Doan

- 403. The specific refractive increment of some purified proteins.** G. E. PERLMANN, AND L. G. LONGSWORTH. Rockefeller Institute for Medical Research. *J. Am. Chem. Soc.*, 70, 8:2719-2724. 1948.

Data were obtained for the quantitative interpretation of the electrophoretic patterns of bovine serum, beta lactoglobulin, egg albumin, human serum and human gamma globulin. By means of a hollow, prismatic cell and the optical equipment of the Tiselius electrophoresis apparatus, the refractive index increments of solutions of the purified proteins were measured as a function of protein concentration, temperature and wavelength of incident light. The changes in specific refractive increment occurring on titration of the protein with alkali, in the presence of neutral salts and after equilibration with buffers, also were determined.

H. J. Peppler

DISEASES

- 404. Brucellosis in industry.** C. F. JORDAN, Iowa State Dept. Health. *Ind. Med.*, 17, 5:176-180. May, 1948.

Consideration is given to the subject of brucellosis as related to industry. Discussion is based on epidemiologic investigation and on a series of 2,405 case reports assembled during the period, 1940-1946, through interest and courtesy of Iowa physicians. Disregarding 25% of the patients who gave no history of contact with animals, attention is directed to the larger number (75%) in Iowa who gave the history of direct contact with farm animals (hogs, cows, sheep, goats) prior to onset of symptoms. Illness in this group is shown to be closely associated with occupations including male farm workers, packing house employees, veterinarians, stock dealers and locker and rendering plant workers. Special consideration is given to the meat packing industry, to measures aimed at eradication of brucellosis in farm animals and at prevention of human illness. With cooperative effort on the part of all interested agencies and individuals, it is certain that continued application of control and preventive measures will reduce the vital hazards of direct contact with infected tissues in proportion as further progress is made toward eradication of brucellosis in farm animals.

Ruth E. L. Berggren

- 405. Brucellosis (undulant fever).** G. R. TUREMAN, JR., 26 W. Williamsburg Road, Richmond, Virginia. *Virginia Med. Monthly*, 75, 1:32-38. Jan., 1948.

From the work of many reliable investigators, brucellosis in man has been shown to be far more prevalent than previously conceived. It is not a self-limited disease running its course in a few days to a few months. Specific therapy, in the form of heat-killed organisms from abortus strains, offers the

greatest hope of cure or improvement in chronic cases. The disease should be considered in all cases of fever of undetermined origin and clinical pictures of a bizarre nature. In making a diagnosis, a combination of all the laboratory tests together with clinical findings should be utilized. The methods of prevention should be stressed, especially in those areas where the disease is commonly recognized. Cases of habitual abortion, in which other causes have been ruled out, should be investigated for brucellosis.

Ruth E. L. Berggren

ICE CREAM

- 406. Dealers profits in dipping bulk ice cream.** V. M. RABUFFO. Ice Cream Trade J., **44**, 8:44-47, 96-100. Aug., 1948.

Tests conducted in New York City have shown that the average ice cream dealer, based on today's costs and selling prices, is making a gross profit of better than 40% without figuring overhead, refrigeration, insurance, etc. Mark-ups on bulk ice cream ranged from 25.2 to 160.4%. Three dealers had a gross of 60% or more, 2 had 50%, 15 had 40%, 15 had 30% and 5 had 20% or more.

Another survey in New York City showed that the average gross profit was 47.8%. The average contents of a 10-quart can was sold to consumers in the following form: 2.875 half pints; 3.5 pints; 0.925 quarts; 14.475 cones; 1.75 dishes; 5.125 sundaes; 13.2 sodas; 9.425 malted and some slight sales going into shakes, floats, banana splits, etc. The average retail selling prices were: half pints, 25 cents; pints, 45; quarts, 90; cones, 8; sundas, 25; dishes, 20; sodas, 20 and malted, 20 cents.

W. H. Martin

- 407. Ice cream cutting and discharging device.** H. A. ALHEIT. U. S. Patent 2,444,486. 8 claims. July 6, 1948. Official Gaz. U. S. Pat. Office, 612, (1):153. 1948.

A scoop which delivers a half sphere of uncompressed ice cream is characterized by a rotating round bowl and a fixed perpendicular member for discharging the molded ice cream.

R. Whitaker

- 408. Milk products and the like containing algin compound.** V. C. E. LE-GLOAHEE. (Assigned to Algin Corp. of America). U. S. Patent 2,445,750. 3 claims. July 27, 1948. Official Gaz. U. S. Pat. Office 612 (4): 921. 1948.

When the acid radical of algin is partially satisfied with calcium and partially with an alkali metal, yielding a final pH of at least 7, it forms a desirable stabilizer for ice cream and other dairy products. The proportion of calcium, as metal, to algin is from 3.1 to 3.5% by weight.

R. Whitaker

PHYSIOLOGY

- 409. Penicillin blood and milk concentrations in the normal cow following parenteral administration.** MARK WELSH, P. H. LANGER, R. L. BURK-

HART, AND C. R. SCHROEDER, Lederle Lab. Div., American Cyanamid Co., Pearl River, N. Y. Science, 108, 2799:185-187. August 20, 1948.

The purpose of this report is not to suggest the parenteral administration of penicillin for the treatment of mastitis. Penicillin can be localized in the udder by intramammary infusions without as rapid loss as when the drug is administered parenterally, and higher concentrations can be achieved on smaller dosage and less frequent administration. However, there is diffusion of penicillin from blood to milk. The total dosage of an antibiotic or a sulfonamide used during the course of treatment is relatively unimportant. However, the dose-time-weight relationship is important.

Ruth E. L. Berggren

410. Physiological action of sodium carboxymethylcellulose on laboratory animals and humans. H. A. SHELANSKI AND A. M. CLARK, Smyth Laboratories, Philadelphia, Pa. Food Research 13, 1:29. Jan.-Feb., 1948.

This rather extensive investigation corroborates previous studies showing that sodium carboxymethyl cellulose (C.M.C.) is harmless physiologically when ingested, even in large amounts, by various animals and humans. These studies presented evidence indicating that "C.M.C." is not absorbed from the intestinal tract, but is almost quantitatively in the feces.

F. J. Doan

MISCELLANEOUS

411. Supporting rack for milk strainers. H. E. MURDOCK. U. S. Patent 2,445,859. 2 claims. July 27, 1948. Official Gaz. U. S. Pat. Office, 612, (4):950. 1948.

A simple wire rack is described for holding a strainer in place in a separator bowl.

R. Whitaker

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

412. **Annual review of biochemistry, Vol. 17.** J. M. LUCK, editor. 801 pp. \$6.00. Annual Reviews, Inc., Stanford, Calif. 1948.

This volume constitutes a worthy successor to the preceding volumes in this series. As usual, the range of the material covered is extensive. The chapters are: Biological oxidations and reductions; Nonoxidative enzymes; chemistry of the carbohydrates; The chemistry of the immunopolysaccharides; X-ray crystallographic studies of compounds of biochemical interest; Chemistry of the lipids; The chemistry of the proteins and amino acids; Nucleoproteins, nucleic acids, and related substances; Carbohydrate metabolism; Lipid metabolism; The metabolism of proteins and amino acids; The metabolism of drugs and toxic substances; Clinical applications of biochemistry; Biochemistry of the hormones; The vitamins; Clinical aspects of vitamins; The biochemistry of carcinogenesis; Biochemistry of the natural pigments; The terpenes (in relation to the biology of *genus pinus*); The alkaloids; Photosynthesis; Mineral nutrition of plants; Plant hormones; Bacterial metabolism; The chemistry of penicillin; Ruminant digestion; Physiological aspects of genetics. Of these 27 chapters, 9 are written by scientists resident in foreign countries.

The large numbers of citations of original literature permit one to refer to the original articles reviewed with a minimum of difficulty. The indexing appears to be done well. The authors and editors are to be commended for making available such a satisfactory resumé of current biochemical literature.

F. E. Nelson

BACTERIOLOGY

413. **Sur l'utilisation de le chloropicrine en laiterie (On the utilization of chloropicrin in the dairy).** J. PIEN, Laiterie des Fermiers Reunis, Paris, France. *Lait*, 28, 271-272: 1-21. Jan.-Feb., 1948.

During 1944 it was proposed by Bertrand (*Compt. rend.*, 219: 230. 1948) that in emergencies milk might be preserved by the addition of chloropicrin. The term microlysatation was proposed for the process, being derived from microlysine, a purified form of chloropicrin. The proposal received approval by the Academy of Medicine and by the Milk and Food Commission for temporary use. Pien reports on the practical aspects of experiments in which treated milk was placed on sale in Paris.

Chloropicrin is not readily soluble in milk and must be incorporated by vigorous agitation. In the trials conducted, concentrated lots were made up and

mixed with milk as received at country receiving stations. This milk, of poor sanitary quality and neither pasteurized nor refrigerated, was placed on sale in Paris. When treated at the rate of 50 mg. per l. the complaints were numerous and vehement due to its instability to boiling. When 80 mg. per l. was used, similar complaints were received and in addition the irritating odor and flavor, even after boiling, were noticed. Sales were discontinued after 12 d.

The rate at which acidities increased in treated milk was studied in the laboratory. Acidity changes were held in check for 2 d. at 25° C. but began to increase after 3-4 d. if the initial contamination was high. Similar results were obtained at 37° C. Upon heating, the milk separated into a smooth, fine curd and a clear upper layer. Total bacterial counts, 24 hr. after the addition of 50 mg./l. to poor quality milk, were large and consisted chiefly of lactic acid producers although large numbers of coliforms, putrifiers and spore-formers were present. The use of 80 mg./l. caused a gradual decline in numbers for 100 hr., while with only 50 mg./l., the numbers doubled in that length of time.

Additional experiments in which chloropicrin was introduced to the milk immediately after milking resulted in good inhibition of bacterial growth. It is concluded, however, that practical difficulties make the use of this disinfectant impossible on the farm.

O. R. Irvine

414. Antibiotics active against bacterial viruses. I. N. ASHESHOV, FRIEDA STRELITZ, AND ELIZABETH HALL, Univ. of Western Ontario, London, Can. Can. J. Pub. Health, 39, 2: 75. Feb., 1948.

Paper discs soaked in substances active against bacteriophages were placed on agar plates flooded with bacteria and phage mixtures. Where the substances showed activity, the disc was surrounded by a zone of normal bacterial growth while the remaining parts of the plate would show confluent clearings of the phage.

An *Aspergillus* was found to produce 2 anti-phage substances, the first an anti-staphylophage substance, acidic in nature, stable between pH 2 and pH 10.0, which withstands heating to 100° C. It has been obtained in crystalline form. Less than 1 part in 10,000,000 by weight will inhibit staphylophage, while staphylococci require concentrations of 1 : 50,000 to 1 : 100,000 before being affected. The second substance from the same mold was active against 3 streptophages out of 12 tried. It is basic and considerably less stable than the anti-staphylophage substance.

O. R. Irvine

415. Method of preparing a vitamin concentrate. N. E. RODGERS, H. L. POLLARD, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,144. 4 claims. Sept. 17, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 434. 1948.

The fermentation of whey and skim milk with *Clostridium acetobutylicum* for the purpose of making a vitamin concentrate, including riboflavin, is improved through the use of a starter which has been transferred a sufficient number of times to give an acidity of at least 4%. All transfers are made within 2 to 5 hours after the maximum acidity is reached.

R. Whitaker

416. Method of enhancing the yield of vitamins in fermentation. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,140. 4 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614**, 2: 433. 1948.

When whey and skim milk are fermented with *Clostridium acetobutylicum*, the vitamin content, particularly riboflavin, is enhanced by the addition of iron and a soluble ammonium salt in a predetermined amount and ratio.

R. Whitaker

417. Fermentation method for preparing a vitamin concentrate. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,141. 3 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614**, 2: 434. 1948.

Essentially the same as abstract no. 415, except iron and a soluble manganese salt are employed.

R. Whitaker

418. Method of improving the yield of riboflavin in fermentation processes. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,142. 4 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614**, 2: 434. 1948.

Essentially the same as abstract no. 415, except iron and zinc are employed.

R. Whitaker

419. Production of riboflavin by fermentation processes. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,141. 2 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614**, 2: 433. 1948.

Essentially the same as abstract no. 415, except iron and a soluble magnesium salt are employed.

R. Whitaker

BUTTER

420. Die Beseitigung des Öl-und Fischgeschmackes der Butter durch pH Regelung. (The removal of oiliness and fishiness in butter by pH regulation.) (English summary.) NIS PETERSEN. Die Milchwissenschaft, **3**, 1: 12-17. 1948.

The authors were unable to correlate the reductase test of milk with oiliness in butter made from the tested milk. Pasture feeding tended to increase incidence of oiliness in butter as compared with feeding hay. Preventive measures against oiliness and fishiness in butter are: (a) mixed feeding ration, (b) high pasteurization temperature, (c) limited washing of butter, (d) low salting of butter, (e) short, rapid working of butter, (f) pH 6-7, (g) protection from light, (h) protection from metal, (i) use of anti-oxidants (wheat germ oil, nordihydroguaiaretic acid, etc.).

I. Peters

- 421. Das Ausölen der Butter. (The oiling-off of butter.)** (English summary.) W. MOHR AND K. BAUR. *Die Milchwissenschaft*, 3, 1: 17-23. 1948.

The oiling-off of butter was measured at 25 and 28° C. Six cubes of butter (1 cubic inch each) were placed on weighed unhardened filter paper (area 5 to 10 times that of the butter cube) and held at 25 to 28° C. for 48 hours, cooled at 10° C. for 30 minutes and the filter paper re-weighed. Butter held at 28° C. was considered to show low oiling-off if 15% or less fat was absorbed by the paper in 48 hours, whereas a 30% loss or more shows high oiling-off. Factors found to decrease oiling-off were: (a) low melting point of the fat, (b) low air content in butter, (c) use of alfa buttermaker as compared with Fritz or churn buttermaker.

I. Peters

- 422. Das physikalische Bild der Butter. (The physical aspect of butter.)** (English summary.) N. KING AND W. FRITZ. *Die Milchwissenschaft*, 3, 1: 2-12; 3, 2: 36-41. 1948.

The authors studied the physical structure of butter microscopically. A known quantity of butter to be examined was stirred into a given volume of butter oil containing 1 to 1.2% octyl alcohol and placed in a counting chamber 0.01 mm. deep, covered with a cover glass and examined under polarized light filtered through a red filter. The fat globules showed a double ring border. Magnification of 400 was used and the temperature of the butter maintained rigidly between 14 to 16° C.

Alfa butter showed the highest per cent of uniform, small, round fat globules with crystals at the border only, whereas machine butter showed larger, slightly flat or irregularly shaped fat globules with crystal formations within the globules. The churn butter took an intermediate position between the alfa and machine butter. The alfa butter had the lowest per cent of free fat and air, followed by the churn and machine butters, respectively.

Butter cubes held at 30° C. for 9 hours and refrigerated over night showed marked difference to cutting resistance. The alfa and machine butter when cut showed a smooth-cut surface, whereas the churn butter was crumbly and difficult to cut. Both the machine and churn butter showed greater melting resistance than did the alfa butter when held at 30° C.

I. Peters

- 423. Production methods and the keeping quality of churning cream.** H. R. THORNTON, R. K. SHAW, AND F. W. WOOD. *Univ. of Alberta, Edmonton, Can. Sci. Agr.*, 28, 9: 377-392. Sept., 1948.

Samples of farm-separated churning cream were collected at 1 creamery and from 2 farms, one producing a high quality cream from machine-drawn milk, while the other ordinarily produced a much lower quality cream. Two additional samples of cream also were secured at the latter farm after the utensils and separator parts had been sterilized. Sub-samples from these were held at temperatures ranging from 40-60° F. for periods up to a month. These

were tested at intervals for off-flavour development, titratable acidity, methylene blue reduction time and bacterial plate count.

Unsterile surfaces with which the cream came in contact, together with storage temperatures above 50° F., were responsible for cream samples dropping from the special grade flavour category. Storage at 50° F. will maintain cream in special grade if delivered twice weekly, while 45° F. storage will maintain this grade for 1 week. The titratable acidity test is of limited value as a means of grading such creams, while the reduction test and bacterial plate count are of no advantage in the routine control of churning creams. Cream may be special grade for churning purposes and yet contain maximum numbers of bacteria.

O. R. Irvine

CHEMISTRY

424. Fosfataseenzymets varmedestruktion. (Destruction of the phosphatase enzyme by heat.) (English summary.) K. P. ANDERSEN, A. M. MADSEN, G. WITTIG, AND H. FAXHOLM. 57. Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1948.

A method for the determination of the laws which govern the effect of heat and time on the phosphatase enzyme was worked out.

A small glass ampule was used for the pasteurization of 1 ml. of milk. The ampule was 100 mm. high, of which 50 mm. was neck. The outside diameter of the neck was 5.0 mm., of the lower part 19 to 20 mm. The thickness of the glass wall was 0.5 mm. The heat conductivity of the glass ampule was 0.026. Heating of 1 ml. of milk 20° C. \pm 0.1° C. would take a calculated 17.1 sec., making it impossible to use direct heat at temperatures so high that the time of destruction would be shorter than time of heating. The cooling of 1 ml. milk from 80 to 60° C. was calculated to take 0.94 sec. in ice water. The destruction of the phosphatase enzyme in this time and at the corresponding temperatures is very insignificant.

These calculations showed it impossible to state the pasteurization time at high temperatures by heat transmission. To overcome this, part of the milk, in which the phosphatase enzyme already was destroyed, was heated to such a temperature that addition of the rest of the milk would bring the temperature down to pasteurization temperature. By churning of raw cream, a buttermilk with a phosphatase activity of 5-6 times that of raw milk was obtained, and this was used where dilution was necessary. The difference in destruction of the phosphatase enzyme in the concentrate and in the corresponding milk was not greater than between 2 different samples of raw milk. The enzyme concentrate was preserved with 1% potassium dichromate in order to carry the whole experiment out with the same concentrate. The preservative caused only a slightly faster destruction of the enzyme as compared to normal milk.

The technic for pasteurizing the milk was that 0.75 ml. of pasteurized milk was preheated to a temperature such that injection of 0.25 ml. of enzyme concentrate at 37° C. would bring the mixture down to the desired holding tem-

perature, following which it was transferred quickly to a water bath of desired temperature and when time was out transferred quickly to ice water. The method was used between 60–80° C. All transfers were made as fast as possible (0.3 sec.). Where the time of destruction was shorter than 1 sec., the transfer to a holding bath was left out.

The speed of reaction of the enzyme was determined at constant temperature ranging from 60–80° C. The results obtained showed that destruction of the phosphatase enzyme is not a first order reaction. The phosphatase enzyme has an optimum stability at its natural pH, compared to pH 6.2 and 7.4. The increase in rate of destruction caused by increase in temperature is found to be in agreement with Arrhenius' formula. Calculations showed that for 5° C. increase in temperature the increase of destruction was 10 times as great, which was in good agreement with results found experimentally. From the results obtained, the time of destruction can be calculated for any pasteurization method.

T. Kristoffersen

425. Trace sugars in milk. C. P. ANANTAKRISHNAM AND B. L. HERRINGTON. Dept. Dairy Industry, Cornell Univ., Ithaca, N. Y. Arch. Biochem., 18, 2: 327–337. 1948.

By means of fractional crystallization, glucose was isolated from dialyzed, de-ionized milk serum. It constitutes the whole of the monoses in milk, and exists in the free state to the extent of 4.08 to 7.58 mg./100 ml. of milk (10 samples). Colostrum milk contains more glucose than normal milk. Immediately after calving, the colostrum of a Holstein cow contained 15 mg. glucose per 100 ml. colostrum, that of a Jersey 12.5 mg./100 ml. The Holstein reached a normal glucose value in 3 days, the Jersey in 10 days after parturition.

H. J. Peppler

426. Some effects of feeding synthetic thyroprotein to dairy cows. C. E. ALLEN, DOROTHY S. DOW, V. S. LOGAN, AND C. D. MACKENZIE. Cent. Exptl. Farm, Ottawa, Can. Sci. Agr., 28, 8: 340–356. Aug., 1948.

In a 3-month trial, a synthetic thyroprotein, "Protomone", containing 3.07% thyroxine by analysis was fed to 3 Holstein and 3 Ayrshire cows at the rate of 15 g. daily for 6 weeks and the effects compared to paired controls. The results include data on the 2-week period preceding treatment and the 4-week period after treatment was completed. Increased milk production occurred in the early part of treatment, the maximum individual increase over the pre-treatment period being 16.7%. All cows, with one exception, declined rapidly subsequent to treatment. Fat tests were increased by from 0.4 to 1.4%, but no significant changes occurred in lactose, total nitrogen, ash or solids-not-fat percentages. Milk yields calculated on a 4% fat basis showed marked increases, the maximum being 41.2%. Body weight losses varying from 7.1 to 13.7% were observed in all cows treated despite the addition of 2 lb. of extra meal per cow per day. Pulse rates were markedly higher for the treatment period but declined after treatment to less than the control animals. No other evidence of ill-health was evident, however.

The need for longer term investigations of the effect of thyroprotein on dairy cattle is apparent before use of this substance can be recommended. The necessity for its strict control to prevent dishonest production records also is pointed out.

O. R. Irvine

427. **Choline content of live stock feeds used in western Canada.** L. W. McELROY, H. A. RIGNEY, AND H. H. DRAPER. Univ. of Alberta, Edmonton, Can. Sci. Agr., 28, 6: 268-271. June, 1948.

The choline content of a number of feeds of different types was found by preparing a solution of extracted choline reineckate in acetone and determining the amount present in an Evelyn photo-electric colorimeter, using filter no. 515. Samples of grain, hay, by-products and commercial poultry feeds were examined and the results recorded. Oats and barley exceeded wheat and corn as sources of choline. Legume hays were found to be better sources than grass hays and the choline content was higher in leafy good coloured samples. Buttermilk powder and whey powder gave values considerably greater than skim milk powder.

O. R. Irvine

FOOD VALUE OF DAIRY PRODUCTS

428. **Discussion on unidentified nutrient.** C. A. CARY AND A. M. HARTMAN. U. S. Dept. of Agr. Certified Milk, 23, 9: 6-9. Sept., 1948.

The existence of an unidentified nutrient X was demonstrated by the improvement in growth of rats when certain feeds were fed in or with the basal ration. In general, the nutrient X was found in leafy foods, liver, beef and pork muscle, egg yolk, whole milk, skim milk, non-fat solids of milk, cheese, crude casein from milk and all milk products containing the crude proteins of milk. It was not found in cereal grains, cereal grain products, yeast and yeast products. Experiments also showed that X may be synthesized by bacteria in the rat. However, the rat's requirement must be supplied in the diet because the synthesis by bacteria occurred only under extraordinary conditions and, therefore, is undependable.

W. S. Mueller

ICE CREAM

429. **Ice cream dipper.** M. F. COSTA. (Assigned to T. N. Benedict Mfg. Co.) U. S. Patent 2,448,863. 1 claim. Sept. 7, 1948. Official Gaz. U. S. Pat. Office, 614, 1: 225. 1948.

An ice cream dipper having a semi-spherical bowl and a scraper blade for discharging the ice cream is described.

R. Whitaker

430. **Frozen food container.** C. R. SYMMES. (Assigned to H. P. Hood and Sons, Inc.) U. S. Patent 2,444,861. 3 claims. July 6, 1948. Official Gaz. U. S. Pat. Office, 612, 1: 248. 1948.

Ice cream and other frozen foods are frozen in a lined cylindrical carton which is so constructed that the product, surrounded by the liner, may be ejected from the container by upward pressure on the bottom. Peeling of the liner is facilitated by tabs on each side of a lengthwise slit in the liner.

R. Whitaker

MILK

- 431. Suspending agent for beverages.** K. W. KARNOOP. (Assigned to Kalva Corp.) U. S. Patent 2,448, 599. 8 claims. Sept. 7, 1948. Official Gaz. U. S. Pat. Office, **614**, 1: 162. 1948.

A water soluble extract of Iridophycus is suitable as a stabilizer for chocolate flavored beverages, such as chocolate milk. R. Whitaker

MISCELLANEOUS

- 432. Cream whipper.** E. C. BRULL. U. S. Patent 2,444,897. 1 claim. July 6, 1948. Official Gaz. U. S. Pat. Office, **612**, 1: 256. 1948.

The ends of 2 rectangular thin flat sheets of bendable metal are fastened together to form an ellipse in side elevation. This dasher is caused to rotate rapidly by means of a vertical shaft through the center. R. Whitaker

- 433. Milking machine.** A. C. BLOEMERS. U. S. Patent 2,445, 904. 7 claims. July 27, 1948. Official Gaz. U. S. Pat. Office, **612**, 4: 960. 1948.

A milking machine which has individual control of the vacuum on each teat cup is described. R. Whitaker

- 434. Milk can drying rack.** E. P. SWINTOSKY. U. S. Patent 2,449,628. 1 claim. Sept. 21, 1948. Official Gaz. U. S. Pat. Office, **614**, 3: 698. 1948.

A simple angle iron rack for holding lids and milk cans in an inverted position to facilitate drying after cleaning is described. R. Whitaker

- 435. Milk can strainer accommodating attachment.** P. P. HANSON. U. S. Patent 2,450,510. 3 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, **615**, 1: 147. 1948.

A rubber gasket is described which fits on milk cans for holding a conventional type milk strainer. Channels between V shaped ribs permit escape of air from the can. R. Whitaker

- 436. Liquid temperaturizing vat.** F. J. McCULLOUGH. U. S. Patent 2,446,054. 4 claims. July 27, 1948. Official Gaz. U. S. Pat. Office, **612**, 4: 999. 1948.

A cylindrical pasteurizing vat for dairy products which provides a built-in heating or cooling device is described. A pump circulates the heating or cooling medium from a sump tank spirally upward between 2 walls containing heating or cooling coils and surrounding the vat. On reaching the top, the medium is sprayed inward against the wall of the vat where it flows downward to the sump to be recirculated. R. Whitaker

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